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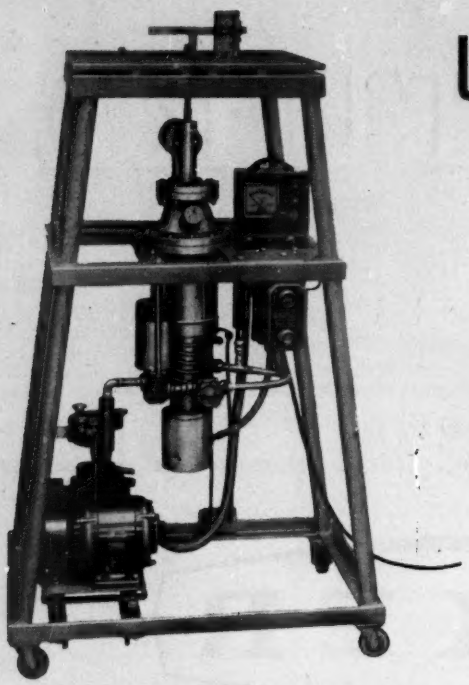

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No. 4678 SATURDAY, JUNE 27, 1959 Vol. 183

CONTENTS

	Page
Expansion of Scientific and Technical Education	1763
John Dalton. By Prof. J. R. Partington, M.B.E.	1765
Applied Analysis in the U.S.S.R. By Prof. T. A. A. Broadbent	1766
The Theory of Practice. By Dr. D. J. Finney, F.R.S.	1766
The American College and the Educated Life. By Dr. Douglas M. Knight	1769
The Role of the Scholar in Society. By Dr. J. W. Cook, F.R.S.	1772
Radioactive Fall-out. By Dr. C. Auerbach, F.R.S.	1773
Obituaries:	
Dr. Jesse P. Greenstein. By Dr. Douglas Hamer; Prof. Alex. Haddow, F.R.S.	1776
Dr. Arthur Walton. By Dr. J. Hammond, F.R.S., and Dr. T. Mann, F.R.S.	1777
Dr. F. Tattersfield, O.B.E. By Dr. C. Potter	1778
Dr. G. K. Groetzinger	1778
Mr. H. Curtis	1778
News and Views	1779
Co-ordination Chemistry. By Dr. L. E. Orgel	1784
The British Coal Utilization Research Association	1786
The Ear Under Water. By Prof. O. Lowenstein, F.R.S.	1787
The Indian Association for the Cultivation of Science	1789
The East African Industrial Research Organization	1789
Part-time Work for Women Graduates	1790
Migration and Dispersal of New Zealand Gannets	1790
Biosynthesis of Cell Constituents from C_2-Compounds. By Dr. H. L. Kornberg and A. M. Gotto; A. G. Callely and Dr. S. Dagley; J. R. Quayle and Dr. D. B. Keech	1791
Constituents of Human Hæmoglobin. By Dr. V. M. Ingram; Makio Murayama and Dr. V. M. Ingram; Dr. Harvey A. Itano and Elizabeth Robinson	1795
Recent Scientific and Technical Books	dcclxx

(Continued overleaf)

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LETTERS TO THE EDITORS

Page

PHYSICAL SCIENCES

Spectrum and Structure of the Free Methylene Radical.—Dr. G. Herzberg, F.R.S., and J. Shoosmith 1801

Removal of Dipolar Broadening of Nuclear Magnetic Resonance Spectra of Solids by Specimen Rotation.—Prof. E. R. Andrew, A. Bradbury and R. G. Eades 1802

Electron Microscopy of Oleophobic Monolayers.—Robert T. Mathieson 1803

Magnetometric Titration: a New Method of Chemical Analysis.—Dr. M. S. Blackie and Dr. V. Gold 1804

A Dose Test for Irradiated Polyethylene.—E. H. Cornish and D. M. Cutting 1804

Secondary Ionization in Humid Air.—Prof. J. M. Meek 1805

RADIOBIOLOGY

Uptake of Strontium by Pasture Plants and its Possible Significance in Relation to the Fall-out of Strontium-90.—Dr. R. Scott Russell and Dr. R. J. Garner 1806

Separation of Strontium-90 from Calcium in Milk.—Dr. N. A. Myers 1807

BIOCHEMISTRY

Position of the Fatty Acids in Cardiolipin.—Dr. Marjorie G. Macfarlane and L. W. Wheeldon . 1808

Differential Response of Human Serum Cholinesterase Types to an Inhibitor in Potato.—Dr. H. Harris and Mary Whittaker 1808

An Alteration in the Excretion of Free Serine in Urine from Irradiated Humans.—Dr. Arthur L. Kretchmar 1809

Mucoprotein of Bronchial Mucus Gel.—J. C. White, P. C. Elmes and W. Whitley . . 1810

Phosvitin in the Serum of the Hen.—Prof. R. H. Common and Chi-Ching Mok 1811

Basic Substances as Synergists for Fat Antioxidants.—H. S. Olcott and E. J. Kuta . . 1812

An Improved Method for preparing Pectate Gels.—Dr. A. M. Paton 1812

Conversion of Caffeic Acid to Esculetin during Paper Chromatography.—Warren L. Butler and H. W. Siegelman 1813

A Proposed Interpretation of Infra-red Spectral Changes occurring upon the Interaction of Polynucleotides.—H. Todd Miles 1814

OPHTHALMOLOGY

Regeneration of the Lens of the Eye in the Rabbit.—D. Stenhouse Stewart and Prof. Paul G. 'Espinasse 1815

PHYSIOLOGY

Induction of Arginase in Rabbit Epithelium by the Shope Rabbit Papilloma Virus.—Prof. Stanfield Rogers 1815

Oral Induction of Leukæmia in Mice.—Bill P. Maduros, Wilma A. Spurrier and Dr. Steven O. Schwartz 1816

Experimental Colloid Goitre produced by Thiouracil.—Dr. R. H. Follis, jun. . . . 1817

Page

Variability of Content of Deoxyribonucleic Acid in *L*-strain Fibroblasts.—Rolla B. Hill, jun., Klaus G. Bensch, Stanley Simbonis and Prof. Donald W. King 1818

Stress Activation of Adrenal Glomerulosa.—Dr. M. Hill, K. Dvořák and M. Pospíšil . . . 1819

Electrical Response to Illumination of the Euphausiid Crustacean Eye.—Elizabeth M. Kampa, Brian P. Boden and Bernard C. Abbott 1820

Melatonin in Peripheral Nerve.—Dr. Aaron B. Lerner, James D. Case, Wataru Mori and Margaret R. Wright 1821

Effect of Enzymes on Mechanical Properties of Tissues.—Margaret L. R. Harkness and R. D. Harkness 1821

Hydrolysis of Lecithin and Lysolecithin by Rumen Micro-organisms of the Sheep.—Dr. R. M. C. Dawson 1822

Bilirubin Metabolism in the Fœtus.—Dr. Rudi Schmid, Sue Buckingham, Gloria A. Mendilla and Lydia Hammaker 1823

Flavone Glucosiduronic Acids in *Scutellaria* sp.: a Correction.—C. A. Marsh 1824

Relationship between Auxins and Nucleic Acid Synthesis in Coleoptile Tissues.—B. B. Biswas and S. P. Sen 1824

BACTERIOLOGY

Neutralization of Bacteriophage by Normal Serum.—Andre J. Toussaint and Louis H. Muschel 1825

Spermine as a Protective Agent against Osmotic Lysis.—J. Mager 1827

Phagocytosis of *Escherichia coli* Protoplasts.—Józef Lisowski, Zbigniew Wieczorek and Miroslaw Kańtoch 1828

Production of Bacterial Mutants with Nitrous Acid.—Dr. Fritz Kaudewitz 1829

BIOLOGY

Effect of Gibberellin on the Termination of Dormancy in Some Seeds.—Prof. Paavo Kallio and Pirkko Piiroinen 1830

Some Unusual Outgrowths from Secondary Follicles in Soay Sheep.—Dr. M. L. Ryder . 1831

Relationship between the Hake Fishery and a Southerly Sub-surface Return Flow below the Peru Current off the Chilean Coast.—Dr. Wilhelm Brandhorst 1832

Life-Span of Rat and Mouse Erythrocytes in the Rat–Mouse Chimera.—Dr. Joan W. Goodman and Dr. L. H. Smith 1833

Assortative Mating and Reciprocal Difference in the Blue–Snow Goose Complex.—Dr. F. G. Cooch and Dr. J. A. Beardmore . . . 1833

Hæmoglobin and the Habitat of the Harpacticoid Copepod *Elaphoidella gracilis* (Sars).—J. Green 1834

Role of the Supra-œsophageal Ganglion during the Early Stages of Caudal Regeneration in Some Errant Polychætes.—Dr. R. B. Clark and M. E. Clark 1834

The Proposed Biological Term 'Pheromone'.—H. S. Micklem; Dr. P. Karlson and Prof. M. Lüscher 1835

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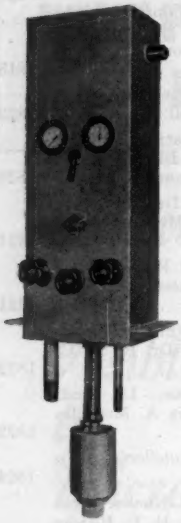

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EXPANSION OF SCIENTIFIC AND TECHNICAL EDUCATION

"ONE determinant of a nation's greatness," writes Dr. Detlev Bronk in his chairman's foreword to the eighth annual report of the U.S. National Science Foundation, "is its courage to choose between the important and the less important. A nation achieves greatness by determined devotion to the things that matter most as it sacrifices the unessential." Dr. Bronk refers particularly to forthcoming debates in the United States on expenditure on scientific teaching and research, and his words are no less applicable to continuing discussion in Britain as to expenditure on education and the balance between that expenditure and the research effort. The account of the Foundation's expanding effort in improving the quality of education in science is indeed the feature of the 1958 annual report which is of most interest to British readers. Educational structures differ widely in the two countries, but the main problems are largely similar, and experience gained in one is likely to have real value in the other, even if methods can rarely be applied without adaptation.

The background to this expansion is well outlined by Dr. A. T. Waterman in his statement as director of the Foundation. Following President Eisenhower's 'messages' of November 1957 and June 1958, particular attention was directed by the Foundation to the development of the capacity of the United States in scientific and technical research, and to its educational resources in science and engineering. Besides the increased responsibilities of the Foundation itself in the field of education, the National Defense Education Act represents a step towards improving the educational system generally; but Dr. Waterman points out that all the measures already adopted are only a beginning. There appears to be no immediate prospect of adequate salaries for school teachers, of adequate school buildings and facilities, or of adequate facilities for recruiting sufficient competent teachers. Plans for dealing with important areas of research are still inadequate, and the urgent problem for science and engineering in the universities of the United States is to secure modern laboratories and research equipment, including rather costly equipment for the larger institutions, and to provide for larger capital research facilities to be used nationally or at regional centres. The training of more scientists also calls for extended laboratories and equipment, and maintenance and operation require expanding funds.

The problems are thus essentially the same as those with which the Advisory Council on Scientific Policy is concerned in Britain, but Dr. Waterman is not satisfied that in the United States they are faced with sufficient determination and realism. Making all allowance for the internal issues which complicate decisions, such as the relative responsibility of the Federal Government and State and local authorities for education, the clash between national policy and individual initiative and aspiration, Dr. Waterman considers that lack of realization and determination on the part of its citizens hinder scientific and educational advance in the United States. A clear conception of national objectives, how they can be achieved, and the citizen's part and responsibility in that task is a first essential, and he suggests that the Federal Government has a first responsibility to see that the problem is fully understood by the people. It must also provide direct support according to carefully devised plans and consider seriously ways and means of increasing substantially funds from other sources.

As broad objectives of national policy, Dr. Waterman puts forward the full development of individual and collective capabilities, and the identification and selection of the fields of effort which merit maximum expenditure of money and man-power. In science, the maximum emphasis should be placed upon the relatively modest needs of basic research so as to discern the possibilities of progress in technology, followed by careful choice of the fields for development that require large capital sums. Those objectives could largely be accepted as valid for Britain, though the distribution of resources and the selection of fields for development would not necessarily be similar, quite apart from any considerations of Anglo-American co-operation.

Reviewing Federal activity in the field of education, the report points out that while Congress has already increased threefold the 1959 appropriation for the Foundation's educational programme (compared with the five-fold increase recommended by the President in January 1958), the National Defense Education Act of 1958 was the first general Federal aid-to-education legislation for ninety-six years, and it re-affirmed the primary educational responsibilities of State and local communities. Apart from providing for loans to students to enable them to attend college, for awards of graduate fellowships and for State grants for vocational programmes for training technicians, under the major administrative responsibility of the Department of Health, Education and Welfare, the Act initiated a Science Information Service in the National Science Foundation to develop new and improved methods for disseminating scientific information.

Some details of the Foundation's educational activities during the year are given in the report. There are in the United States some 140,000 high-school teachers of science and mathematics, though a significant proportion are not adequately trained, and in the spring of 1958, 16,000 of them applied for admission to the summer schools which the Foundation sponsored. Of these, 6,300 teachers received support from the Foundation grants at summer schools averaging seven weeks in duration. Grants were also made to nineteen universities for similar

schools in science and mathematics during the school year 1958–59, in which 925 teachers were trained, and for 1958–59 about thirty summer schools are planned. About 3,000 high-school teachers attended other 'schools' offering courses out of school hours, and for 1959–60 the number of such institutes supported by the Foundation is to be increased from 86 to about 200. The Foundation also proposes to offer about 550 fellowships for graduate teaching assistants for the summer of 1959, to enable them to pursue, full-time, their own study programmes during the summer months, and about 750 science fellowships for secondary school teachers of science and mathematics who hold bachelor's degrees to enable them to pursue a personal study programme over one to three summers.

Besides these programmes attempting to improve the qualifications of existing teachers and to shorten the time in which qualifications can be obtained, the Foundation is trying out new methods of increasing the understanding of science by young people. These are of three types. Some seek to enlist the interest in and understanding of science, mathematics and engineering by students at all levels. These include a travelling high-school science library programme, a travelling science demonstration lecture programme, particularly in such subjects as solar radiation, atomic structure, nuclear reactions and space travel (a programme which enables distinguished scientists to visit small colleges and universities for several days), a science club project and a summer training programme for secondary school students. The second type comprises a variety of teacher-training projects, and the third stresses studies of the content of science curricula in secondary schools, in which new and imaginative approaches to instruction in science have resulted from the judgment and experience of scientists and teachers, including the development of an entirely new course in physics for the high-schools.

Some of these programmes are extensions or developments of projects on which the National Science Foundation had already embarked, and parallel activities are indeed to be found in Great Britain. The Minister of Education, for example, has already recognized the need for examining our methods of teaching science and our curricula with fresh minds. The Advisory Council on Scientific Policy is known to be aware of these problems, and an important session at the Home Universities Conference last December on standards of teaching and techniques and the supervision of students in universities showed that the universities are already considering what can be done, quite apart from the implications of the forthcoming expansion. To a limited extent, the opportunities which new techniques, such as sound broadcasting and television may provide, are being explored, although there is nothing exactly parallel to a project supported by the National Science Foundation and sponsored by the American Institute of Biological Services which seeks to evaluate the effectiveness of the use of experimental colour television films in biology.

The annual report of the National Science Foundation is thus a welcome reminder of possibilities in meeting the shortage of science teachers and in expanding our output of scientists and technologists. It does more than point to possibilities which may here have been overlooked or been insufficiently examined. It sets the whole problem in its proper perspective and emphasizes the need for looking at education as a whole and for dealing with it with real determination, urgency and vision.

On that there are two points to be made. A first priority is the creation of a wide public understanding of all the issues involved; this is a task to which professional associations generally, and not those of teachers alone, should be addressing themselves. The British Association is already supporting important projects to this end, as the American Association for the Advancement of Science has done, and some of the professional organizations are already taking an increasing interest in the training of those entering their own professions. It should not be a difficult step from such work to participation in the task of developing a wider public understanding of the implications of education generally.

Secondly, while the American effort in the improvement of the quality of scientific and technical education and the expansion of its scale, vastly exceeds the present effort in Britain, it is important to remember that mere expansion itself is not enough. Expenditure on education in Britain must be appropriate to national needs and resources, and conditioned by them and by our ideals and traditions, not by those of other countries, however closely associated. Moreover, it is at least as important to see that the most effective use is made of expenditure as to see that the expenditure is adequate. As in research, there is real danger that full use will not be made of existing facilities and expenditure through failure to provide the essential equipment, aids and supporting technicians.

In this connexion, the discussion at the Home Universities Conference* is suggestive. Prof. R. J. Pumphrey challenged the validity of the existing lecture system, and he pleaded for wider use of libraries and reading and the more generous provision of such facilities; and although others, like Prof. J. T. Brindoff, did not agree that the university lecture had outlived its usefulness, there was strong support for his plea for expansion of the university library, though it was also pointed out that the student has yet to learn the art of reading in a library. It was clear enough that fresh thought is already being given to existing methods and techniques, and that a comparatively minor increase of expenditure at particular points would enhance the present efficiency of both teaching and research. Indeed, Prof. B. Bleaney showed that there is very real danger so far as physics is concerned, unless larger Treasury grants are forthcoming. It was no

* Report of the Proceedings of the 1958 Home Universities Conference convened by the Committee of Vice-Chancellors and Principals and held in the William Beveridge Hall, University of London Senate House, 12th and 13th December, 1958. Pp. 120. (London: Association of Universities of the British Commonwealth, 1959.) 5s.

less apparent, however, that if in the expansion of education in Britain already proceeding the full benefits are to accrue, to the individual and to the community, there must be clear conceptions as to the exact functions of our educational institutions, the purposes they are to serve and their relations to one another. These must be in harmony and the resources placed at their disposal appropriate to the function to be served, if we are to avoid either waste of effort and resources or to achieve such a standard of education and supply of trained men and women as Britain needs to use the opportunities that confront us in this age of rapid technological change.

JOHN DALTON

The Biographical Approach to John Dalton

By Frank Greenaway. *Memoirs and Proceedings of the Manchester Literary and Philosophical Society*, Vol. 100, 1958–59. Pp. vi + 98 + 11 plates. (Manchester: Manchester Literary and Philosophical Society, 1958.) 21*s*.

THE hundredth volume of the *Memoirs and Proceedings* of the Manchester Literary and Philosophical Society consists of a memoir by Frank Greenaway on "The Biographical Approach to John Dalton". It deals with the older biographies written by Henry, Angus Smith, Lonsdale, and Roscoe, and also gives, for the first time, a connected account of the newer work of Roscoe and Harden, Meldrum, and Gee, Coward, and Harden, as well as of some later publications.

This essay is very interesting, and a good deal of painstaking work has evidently gone into it; but it fails, in my opinion, to do justice to Dalton. It includes too much which is irrelevant and omits too much which is essential to a proper understanding of the place of Dalton in the history of chemistry. Some justification for the last statement can first be given.

It has often been said that before the enunciation of his atomic theory Dalton did little or nothing of importance. In fact, Dalton had published work on the theory of mixed gases and the state of water vapour in the atmosphere, on the conduction of heat in liquids, on the vapour pressure of water, and on the expansion of gases by heat; and if he had done nothing else he did, and would still, rank high. Mr. Greenaway says that in his early meteorological work, "Dalton slipped into a groove of study which led him to a position he never fully understood". It led him to the discoveries mentioned above and to ideas which, unless we knew that they were first published by Dalton and were initially received with incredulity, we might think are self-evident.

It has also often been said that after the publication of his atomic theory Dalton did no work of importance; he was, says Mr. Greenaway, "an anachronism". Yet some of his later work, such as that on the combustion of hydrocarbons, on the vapour density of ether, and on the isomeride of ethylene in coal gas is really important.

Another old error which is repeated is that Dalton was a coarse and inaccurate experimenter. His work on gas analysis, which (as everybody who has done any knows) is difficult, is quite exceptionally good, and although Mr. Greenaway dismisses the third volume (II, ii) of the "New System" in a word or two as of no interest, it contains some surprisingly accurate work on the equivalents of metals which has been pointed out by Trengrove. A mention of Dalton's "undeserved reputation as a chemist" is unnecessarily severe on the judgment of contemporary chemists.

Dalton's early work on the aurora is given but it is not mentioned that Faraday later proposed the same theory without naming Dalton; no reference is made to Dalton's later experiments on the composition of the higher atmosphere and his explanation of the results, quite recently proposed again as original.

Dalton did not read many books, but the suggestion that "his often-quoted claim to be independent of them may well have been an impromptu attempt to conceal a feeling of inferiority in the face of those who were more accustomed to them", is one of those random guesses which sometimes pass for biography. One of the books Dalton read in his early period is Bishop Watson's "Essays", on which F. Sherwood Taylor's over-clever statement that it could have been written in a year and "smells more of the lamp than the laboratory" is quoted. This book (in five volumes) records Watson's visits to mines and factories extending over many years, and contains some extensive research of his own which is known to physical chemists. Watson's work on gunpowder is also well known. This sort of history simply will not do.

Dalton's mentality seems to have been relatively straightforward as compared with personalities such as Newton, Priestley and Lavoisier. Mr. Greenaway gives an anecdote (p. 7) about Granville (who is otherwise little known) revisiting Dalton in old age and finding him busy trying to loosen a stuck glass stopper. Dalton gently resisted an attempt to assist and finally released the stopper himself. Then, although obviously busy, he had a few courteous words with his unrecognized visitor and got rid of him. This was typical of Dalton, but all Mr. Greenaway gets out of it is that "there was a little glow still in the grey ash of the man".

The section on the origin of Dalton's atomic theory is one of the best, but it too fails to bring out some important matters. Mr. Greenaway thinks that earlier writers have been inconsistent in saying that the origin goes back to Newton while Dalton used a theory of heat different from Newton's. A little might have been said about Newton's own use of this theory of heat, and why Dalton adopted a different one. The influence of Lavoisier's "Traité" could have been considered, and particularly how Dalton's criticism of an erroneous theory of mixed gases contained in it was related to the development of his own theory.

The final section of the essay is a rather confused assembly of some recent pronouncements on the philosophy of making discoveries; it is not easily understood.

If I were asked to hazard an opinion I would say that Dalton has been too much for many of his biographers; the most successful of these was probably Roscoe, largely because he was a chemist, had read Dalton carefully, and had a good knowledge of the state of physics and chemistry when Dalton began his work. The same qualifications are needed in a future biographer of Dalton, who will have the advantage of new knowledge which has accumulated since Roscoe wrote.

Mr. Greenaway's essay gives a good if incomplete summary of this new knowledge, and he has added a few new morsels—no mean achievement in the field, since much of the material has been lost. He deserves thanks for what he has done. J. R. PARTINGTON

APPLIED ANALYSIS IN THE U.S.S.R.

Approximate Methods of Higher Analysis

By L. V. Kantorovich and V. I. Krylov. Translated from the Russian by Curtis D. Benster. Pp. xv+681. (Groningen: P. Noordhoff, Ltd., 1958.) n.p.

THE first edition of this book appeared in 1936, as "Methods for the Approximate Solution of Partial Differential Equations"; it dealt with boundary value problems for linear equations. The title was changed to the present one for the second edition (1941), on the inclusion of an extended treatment of conformal mapping, and an account of the numerical solution of integral equations. The present translation is from the 1952 edition,, which does not differ greatly from the earlier editions: the authors promise a complete revision which shall take account of the substantial progress made since 1941, when no doubt the use of linear operators and function spaces, for example, will find a place. In the interim, a translation is most welcome, since the book as it stands is probably alone in its class, yet for linguistic reasons many of us have been debarred from immediate knowledge of its richness of content. In spite of an increasing flow of translated books and periodicals, it is not easy to acquire a full insight into the rapid growth of Soviet mathematics, though F. J. Weyl, writing from the U.S. Office of Naval Research, measures the will to do so by his remark: "It is a unique distinction of the mathematical community to have never been taken in by the myth, now generally shattered, that Russian science merely followed the lead of the West—at a respectful distance".

The book in each of its main sections exhibits the intention of pressing the processes of pure mathematics beyond the domain of form and theory to the region of numerical calculation, to numerical approximations accompanied by estimation of error. Methods are illustrated by examples from various fields of applied mathematics, carried out to detailed numerical conclusions with evaluations of bounds to errors. The first section deals with solutions of Laplace's equation by infinite series, including Fourier series, with paragraphs on methods of accelerating the convergence. The second section discusses numerical methods for solving integral equations of Fredholm's type, with some emphasis on the 1930 method of N. M. Krylov and N. N. Bogoliubov, illustrated by solving the Dirichlet problem for an elliptic boundary. In the third section, the method of nets, which replaces a partial differential equation by a set of finite difference equations, is expounded with attention to the convergence of the process and the estimation of error. Rather more advanced theory begins in the fourth section, which studies variational methods: the Rayleigh-Ritz method, first fully elucidated by N. M. Krylov, and the related but somewhat simpler and more general method of Galerkin, are fully explained and exemplified. The fifth and sixth sections form a compact treatise of 250 pages on the theory and applications of conformal mapping; the reader is supposed to know the elementary ideas as far as Riemann's theorem on the existence of a conformal mapping between two simply connected domains. The boundary-value problems of Dirichlet and Neumann, and for the biharmonic function, receive thorough treatment. There are numerous examples, though, for the biharmonic problem, the reader is naturally referred to the many and varied applications of the method made by Muskhelishvili in his excellent book on elasticity. It may be noted that Muskhelishvili refers to Kantorovich and Krylov as a classic work on the application of complex variable methods, and the two books resemble one another closely in spirit and in technique. The final section gives Schwarz's method of solving Dirichlet's problem for a complicated domain by combining solutions for simpler domains: the method is illustrated by the complete solution for an L-shaped domain.

As a compendium of methods available in 1940, and as an instance of what can be done by skilfully combining general theory with numerical analysis, this is a noteworthy volume. It should be interesting to see how future revisions are modified by the increasing dominance of the automatic computer. The translation, by C. R. Benster, is fluent: the bibliography unfortunately makes no serious attempt to include non-Russian material. T. A. A. Broadbent

THE THEORY OF PRACTICE

Planning of Experiments

By Dr. D. R. Cox. (Wiley Publication in Applied Statistics.) Pp. vii+308. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1958.) 60*s*. net.

DR. D. R. COX succeeds well in his aim of writing an introduction to modern thought on the design of experiments free from the statistical and mathematical technicalities that often discourage the experimental scientist from study of a subject so inportant to his own work. He says much about the nature of computations required in the analysis of experimental results without presenting one analysis of variance table. He displays a wide range of standard experimental designs, and discusses their uses, while saying little about how to construct them or about mathematical theorems relating to their existence.

Although an increasing number of scientists has sufficient knowledge of statistical practice to learn about experimental design more intensively than this approach permits, undoubtedly many others will profit from it. The book could be an admirable introduction to statistical ideas for a biological or industrial scientist; from it he would learn little about the standard procedures of statistical analysis, such as are to be found in most elementary text-books, but he would acquire something of greater practical value, an appreciation of the manner in which the design of an experiment affects the precision of the results and the efficiency with which the experimenter's time and resources are used.

Dr. Cox's book is far more than an introductory text. With the exception of Davies, probably no writer on design has given comparable attention to questions of general planning, such as the choice of treatments and levels for testing, of experimental units, and of measurements. Anyone concerned with experimental statistics can benefit from studying, for instance, the sections on types of factors and interactions and the choice of factors for inclusion in an experiment. An excellent and varied collection of examples will make the book also of value to the teacher.

The only serious blemish is the conversion of 6.25 dollars into a British price of 60 shillings.

D. J. Finney

Bertil Hanström

Zoological Papers in honour of his sixty-fifth Birthday, November 20th, 1956. Edited by Karl Georg Wingstrand. Pp. 312. (Lund: Zoological Institute, 1956.) 50 Sw. Kr.

SCRUTINY of these papers soon reveals that they mark more than a formal occasion. In offering them to Prof. Bertil Hanström, the contributors have taken obvious pleasure in expressing their gratitude to him " . . . as a teacher and leader of the Zoological Institute in Lund".

Prof. Hanström's first paper was published in 1915, and it dealt with the birds of the island of Öland. While his subsequent work has been mainly concerned with nervous systems, particularly with neurosecretion, Hanström still finds time for field studies. It is thus not surprising that Lund is now an important centre for systematic, zoogeographical and ecological work.

Hanström's interests are clearly reflected in this series of papers. As might be expected, there are several papers on neural and neurosecretory systems (concerning insects, chilopods, crustaceans, cephalopods and the toad, *Bufo bufo*). Others deal with the systematics and relationships of crustaceans, ecology and cytology. The range of these papers shows clearly that zoology is in a flourishing state at Lund.

The contributions are nicely produced and bound, the design for the cover being particularly appropriate.

N. B. MARSHALL

Diagnostic Performance Tests

A Manual for Use with Adults. By Boris Semeonoff and Eric Trist. Pp. xv+176+4 plates. (London: Tavistock Publications, 1958.) 32*s*. net.

AS is well known, intelligence tests were evolved in order to detect and assess educationally subnormal schoolchildren. Their intention was to sample mental growth in its various aspects and to provide reliable standards of performance in relation to age. By and large they succeeded admirably. In the case of adults, on the other hand, mental testing has proved altogether less satisfactory. Although a wide variety of tests purporting to measure adult intelligence exists, it is seldom clear precisely what is being measured or how much confidence can be placed in the results. In consequence, the use of intelligence tests with adults has been viewed—in Britain at least—with understandable reserve.

The methods of testing intelligence described in this book have an unusual history. In 1940, Mr. Trist was concerned to develop some tests of intellectual deterioration due to injury or disease of the brain. In the light of the work of Goldstein and others, it had become plain that such deterioration is particularly liable to show itself in performances involving abstraction, spatial analysis and sequential learning. Mr. Trist was therefore led to devise a small battery of tests well adapted to reveal defects in these fields. But it soon became clear not only that extensive control data were necessary but that the techniques might have important application to the assessment of healthy individuals. At this point Dr. Semeonoff entered the picture and was able to carry the work very much further. Indeed, the present book is very largely the outcome of his prolonged and patient endeavour.

The performance tests reported here have the advantage first, that they make little demand upon linguistic sophistication; and secondly, that they permit some evaluation of important aspects of personality. Indeed, the emphasis throughout is upon assessment of the individual as a whole rather than upon some arbitrary dimension of intellect or temperament. In consequence, these tests may be expected to find application not only in clinical psychology but in any field of personnel selection in which considerations of temperament are relevant.

This battery of tests is perhaps the first to be evolved in part at least on a neurological basis. If it be conceded that the brain has at any rate something to do with intelligence, this would seem a notable—if belated—achievement.

O. L. ZANGWILL

A Study of Blackbirds

By Dr. D. W. Snow. Pp. 192. (London: George Allen and Unwin, Ltd., 1958.) 21*s*. net.

BEFORE the natural history of a wild species can be understood it must be studied from more than one point of view—only when behaviour, ecology, systematic position and other aspects of its biology are inter-related can a full picture be obtained. This book describes a study in which much progress has been made in this direction. Based on four years work in the neighbourhood of the Botanic Garden, Oxford, it presents intimate details of the history of a small community of blackbirds, many of which were colour-ringed. Although written in non-technical language (much of the material has also been published in scientific journals), the author places his observations against a wide background of biological principles, and thus provides material both for the general ornithologist and for professional biologists.

As well as descriptions of the life-history, displays and vocalizations of the blackbird, many interesting facts which could have come only from a close study of colour-ringed birds are presented—for example, the pairs which are sometimes formed in winter are only temporary affairs; and male and female feed different individual young after the brood has left the nest.

Perhaps the most interesting chapters are those which deal with population problems. In particular, a contrast is drawn between garden and woodland populations. The former are dense, with a high breeding success, and produce an excess of young birds which do not disperse before settling. Under these conditions, territorial behaviour limits breeding density. Woodland, on the other hand, is sparsely populated, and breeding success is low; population density is probably controlled largely by survival in the preceding winter.

R. A. HINDE

Proceedings of a Symposium on Insect and Food-plant

Wageningen, May 27–29, 1957, under the auspices of the Comité Européen de Zoologie Agricole (C.E.Z.A.) and the Agricultural University, Wageningen. Pp. 118. (Wageningen: Landbouwhoogeschool Laboratorium voor Entomologie, Berg 37, 1958.) 5 florins.

SYMPOSIA are becoming increasingly used as a means of communication between scientists; subsequent publication of the reviews and discussions often go far to help the research worker to keep abreast of the latest ideas and results of others. According to the preface of the proceedings of this symposium, the organizers had hoped to obtain during the discussions "a synthetic view of the whole complex of insect – host-plant interactions". Although this aim was, unfortunately, not achieved,

the articles are a useful survey of recent work which every agricultural entomologist will want to have by him.

The symposium was held under the auspices of the Comité Européen de Zoologie Agricole and the Agricultural University, Wageningen, under the presidency of Prof. B. Trouvelot. The contributors came from six different European countries and one came from Canada; their articles are in English, French and German, most of which have a summary in one of these languages. Ten of the fourteen articles have already appeared in Vol. 1 of the new journal, *Entomologia experimentalis et applicata* (*Nature*, 181, 1040; 1958).

The emphasis of the symposium was on food-plant selection and the resistance of host-plants to phytophagous insects. Six articles deal particularly with the Colorado potato beetle which has received much attention from European workers in whose countries it is a serious pest; work on host-plant resistance to the beetle among the Solanaceae and on the behaviour of the larva is reviewed. Two papers are concerned with aphids, and one, by a botanist, reviews the related subject of phloem sieve tube sap. One contributor discusses the significance of the salivary secretions of 'plant-sucking' Hemiptera, and another, in a brief summary (in German), deals with his work on the choice of food by certain lepidopterous pests. Two articles on the potato-root eel-worm are included on the grounds that the problems of the nematologist are similar to those of the agricultural entomologist, but an article on the dietetics of a blowfly appears to be somewhat out of place. C. J. Banks

Instrument Encyclopedia

Compiled by Edward W. Battey. Pp. xix+292. (London: The Herbert Publishing Co., Ltd.) 63*s*. net.

THE purpose of this book is defined in the preface as follows: "... to provide a concise reference to the materials and equipment used and produced in the Instrument Industry ... and to define the terms employed and required by instrument users in every industry. The Encyclopedia is not in any sense intended as a text-book. ..." The compiler has realized these aims remarkably well.

The book starts with a detailed index and a short but well-planned encyclopædic section explaining meanings of technical terms. It includes some of the near slang expressions which have found general use, and references are given, where appropriate, to British Standards specifications. The remainder of the book consists of alphabetical lists of manufacturers, complete with their addresses and telephone numbers, a list of overseas agents, a catalogue of trade names and an extremely full buyers' guide. This last section is the largest and perhaps the most useful. It permits one to discover the manufacturers of any specific instrument or component, and is divided into eight sections (Laboratory, Medical and Research; Optical and Ophthalmic; Electronic and Nucleonic; Electrical; Engineering; Automation and Process Control; Accessories and Components; Nautical, Aeronautical and Surveying.)

In order to make some kind of assessment of the completeness of the information a number of 'spot tests' have been applied, and the "Encyclopedia" survived most of them with flying colours. It defeated many attempts to think of lesser known firms or instruments which, though not included, should legitimately come within its scope.

The only real criticism to be made is of the title. One usually thinks of an encyclopædia as a collection of explanatory information: the actual encyclopædic section of this book is only 28 pages long and perhaps it would have been wiser to choose a title more in keeping with the remaining 264 pages. However, it will undoubtedly prove a most valuable work of reference, particularly for those needing to use apparatus and instruments not closely allied to their own field of work. C. A. Taylor

Dictionary of Physics

Compiled and Edited by H. J. Gray. Pp. x+544. (London and New York: Longmans, Green and Co., Ltd., 1958.) 84*s*. net.

THERE are obvious difficulties in reviewing a dictionary since the reviewer can scarcely be expected to read it from cover to cover. But 'spot' checks in several different branches of physics showed very few weaknesses; only a few items sought (synchrocyclotron, photo multiplier, Fermi surface) were not found. In general, the definitions are clear and concise, and references are given for follow-up reading. Perhaps the policy of giving only the earliest references is unwise, even if historically they are the most important.

A few mistakes were found: the diagram of the spherometer is described as that of the planimeter; the last sentence of the entry for Laue is wrong; a sign convention in optics is called the 'real position' convention. But on the whole the number of such mistakes appears to be small. The reviewer is glad to note that the dictionary insists on the correct spelling for Abbe (not Abbé) and is not caught out by Kirchhoff; it also ascribes Bragg's law to the correct Bragg.

The dictionary may thus be described as an extremely useful work. It is obviously not perfect; but it will form a handy work of reference for any physicist. H. Lipson

Zirconium

By Dr. G. L. Miller. Second edition. (Metallurgy of the Rarer Metals, No. 2.) Pp. xxi + 548. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1957.) 70*s*.

FIRST published in 1954, this important work of reference on the manufacture and properties of zirconium has been considerably re-written and enlarged with an increase in the number of pages from 374 to 535. The price of the book has also increased from 45*s*. to 70*s*.

Dr. Miller has made some reconstruction to the text and the arrangement of the matter and has taken advantage of relaxation of classified information in various directions, and added a considerable amount of new data.

The principal additions are in the description of the Kroll process, in the description of the thermal properties and the tensile properties of zirconium, and in the description of the mechanical properties of certain zirconium alloys. The section on corrosion of zirconium by liquid metals has been expanded substantially and the number of pages devoted to melting practices has been doubled. There have also been additions in connexion with the fabrication processes for zirconium and in the description of the nitrides, carbides, borides and silicides.

This book represents the best compilation of information on this subject at the present time.

W. D. Jones

THE AMERICAN COLLEGE AND THE EDUCATED LIFE*

By Dr. DOUGLAS M. KNIGHT
President of Lawrence College, Appleton, Wisconsin

THE Rockefeller Institute lends and can lend unique distinction to our image of the educated man; but that image could not properly exist without a widespread and deeply rooted sense of what goes into his creation, encouragement, and nurture. The community of graduate scholars at its finest is not possible unless there stands prior to it the community of those who may some day be scholars.

The mandate to create a climate for liberal learning has been alive in most undergraduate colleges since their beginning. Their frontier setting, their financial struggles, at times their narrow sectarianism—none of these things should blind us to the fact that their very foundation in the midst of so much uncertainty was a major act of faith, and faith in the same qualities of inquiry, judgment, commitment which two hundred years earlier had led to the founding of the country's oldest college. Fortunately for American intellectual development as a nation, many of the nineteenth-century undergraduate colleges have remained true to a version, at least, of their original duty. To say so implies no criticism of those which have become world universities during the last century, but a Harvard, a Yale (or a Rockefeller Institute, for that matter) owes much of its opportunity for great achievement to a group of small, less privileged institutions which in the last hundred years have developed the art, the science, and the community of distinguished undergraduate education. The context of the houses at Harvard and the residential colleges at Yale is not merely the tradition of Oxford and Cambridge; it is equally the tradition of the American college.

I sometimes hear it said that in the harassed world of to-day and to-morrow the undergraduate college will have a precarious and uncertain time; those who make the remark do not know the history of these colleges, which in a sense has always been precarious; nor do they remember that any great cause must have elements of the precarious and uncertain about it. The history of the American college at its best is a history of steadily increasing strength, of increase in intellectual range and sophistication without the sacrifice of coherence, of increase in duty to the country without the sacrifice of local obligation.

The gravest danger in the years ahead (and if it is a danger to the colleges it will be an equal menace to the great communities of graduate study) is not the danger of financial bankruptcy or depleted faculty. The greatest danger will come from the more subtle and more pervasive issues which involve our whole society—the steady shift towards a national uniformity in culture, the equally steady diffusion of residential communities, the explosive developments of knowledge which often outdate our perception before we have time to master it. These shifts will stand behind the real crises of undergraduate education in the years just ahead of us. These are more critical issues for the college than for the university, simply because the college depends for its life on the real and daily community of those who learn, and equally on the orderly assimilation of the major disciplines and the major modes of understanding. A great university to-day often assumes (for better or worse) that it is not one community but many; it assumes equally that it has a primary obligation to the growing edge of knowledge in a hundred fields, rather than to the full assimilation of knowledge either within these fields or between them. Such assumptions would be fatal for a college; it must face instead a question in some ways more complex than the university question. It must maintain the community of learning both in idea and in fact; it must affirm and sustain the steady growth of knowledge, while equally it must maintain the coherence of knowledge. Both in its sense of community and its approach to learning, it must avoid the cold death of being academically outdated on one hand, or the fiery end of being intellectually fragmented and shattered on the other. Unless this balance of forces can be sustained, the distinguished liberal colleges will obviously suffer in the next twenty-five years; and if they do fail in their attempt at coherence, the damage to the university and the graduate research centre will be greater than most of us realize.

The hope and destiny of the learned life cannot be confined to one type of institution, or one advanced level of performance, no matter how superb. The scholar who cannot depend upon a climate of respect in his society will not long be able to guarantee his scholarship: and since the live interchange between the scholarly life and the whole life of society occurs nowhere else so effectively as in the undergraduate years, it may not be totally arrogant to say that the future of the Rockefeller Institute rests partially in the power of a hundred liberal colleges not only to provide graduate fellows but also to maintain the climate of learning in the United States—even for and in those who themselves will never be learned men or women.

If this is so, however, then any college of stature is faced with a triple responsibility: first, for the education of those who are destined to be scholars; secondly, to the sense of coherence in knowledge without which true learning soon becomes impossible; and thirdly, to the national climate of respect for learning which must constantly increase if the learned life is to keep pace with the terrifying growth of American society. How are these things being done, and how are they to be done in the years ahead?

Two chief means are available to the college: the first grows from a formal pattern of study, the second from the living fact of an academic community. I assume that there is no uniquely effective curriculum, and no perfect community. If we look at the ends of formal education rather than its means, however, we do find a certain uniqueness of purpose in the liberal college. It assumes above all that the great traditions of knowledge and its emergent patterns have much to do with one another; that the study of Plato, for example, and mathematical

* Substance of an address delivered on May 21 at the Rockefeller Institute Convocation for Conferring of Degrees and Dedication of New Buildings (see also *Nature*, 183, 1629; 1959).

theory of government, and contemporary nationalism in India may have something crucial in common. If it is to be worth anything, this sense of continuity must look with an equal eye on the genuinely new and the permanently valid; it must represent the true and persistent relationship of many minds at many times, while it maintains its final allegiance and its deepest reverence for the timeless moment of discovery which flames only in the individual mind.

To say this is, of course, to imply that the curriculum of the liberal college cannot be regarded as an end in itself; whatever its formal content, whatever the means it uses to guarantee the continuum of knowledge, a course of study has its meaning only if it assumes life in the individual; it is the single person who is the true unit of education—not always a person alone, but still an individual, no matter how many times he is multiplied in his community. The great curse of size and mass in education is that they blind us to the end, and lead us instead to talk endlessly about the process. The process of education is no substitute for the educated man; in a sense the greatest responsibility of liberal education is to keep its process simple enough so that the event of true education can still occur.

If this event of education involves the individual, it also involves something very special about the individual, that is, his power to create. It is a great pity that the nineteenth-century encouraged its artists to pre-empt the language and idea of creativity; for there is no difference except one of mode between true insight about the order of protein molecules and that true insight of the poet which James Joyce describes as "his own rare thoughts, a chemistry of stars". There is no difference, except one of degree, between the subtle and distinguished perception which adult intellectuals are capable of and the first fumbling recognition by a child that the black lines on a page somehow represent reality.

In all these cases the creative mind is proving itself, alive in the individual and working with the continuum of knowledge, and it is actually this living triad of individual, creative insight and tradition of knowledge that makes the liberal college a major force in the intellectual life of our time. Any one of the three, or any two, would fail to provide that explosive encounter with the intellectual life which is the hallmark of true education as opposed to dead learning or technical discipline.

This encounter in turn both creates and is created by a particular kind of community. Historically, of course, the undergraduate college had about it the inevitable and simple order of Plato's academy (often, alas, without Plato); but the thrust of our society has put upon it a duty not imagined a century ago. As the great cities have sucked in millions of people, and then flung them out again to spawn in suburbia; as transportation has made the means of movement easy and the ends more and more obscure; as migration has become the norm of social life and not just a function of the frontier—as all these things have taken place, we have found ourselves confronting, not the regional social order that sustained us a century ago, but a national and indeed supra-national order within which we must somehow find a path. Regional order gave firm, even if narrow, support to the individual; but our struggling world order is a threat instead. It forces upon each of us the necessity to define ourselves, to establish our powers and our commitments, and then to use our talents in the service of a society that does not yet exist. We must realize that to-day we shall not find encouragement toward intellectual excellence anywhere unless we find it in academic community. Where once we could share the responsibility with many other aspects of society now we must carry it ourselves in the undergraduate colleges and the great graduate centres of learning.

As a result we have substituted in education a new frontier for the old, a frontier of the mind to replace our old frontier of physical expansion. To put the matter another way, where colleges and universities once reflected their culture, they must now create, establish and confirm it. As our society has cast off all sense of its own coherence, we have assumed the burden of creating coherence again, and often in the face of scorn or even angry opposition. The intellectual obligation of the college is equally in our future a moral and social obligation.

This obligation will be met in many ways, of course, but above all through the unique power of the undergraduate college to bring together qualities of mind and person which are often kept apart by the rest of our society. I shall mention only three of these complex qualities, and I shall represent them by a pair of what I might call 'related opposites': first, knowledge and immediate experience; secondly, tolerance and commitment; thirdly, individuality and community. In each case one half of the equation—knowledge, or tolerance, or individuality—is commonly regarded by our society as somehow less significant than the other half—immediate experience, or commitment, or communal life. It is the insistent claim of the best education, however, that we cannot participate in the immediacies, the commitments, the communities of our world until we understand them; but it is our equal claim, of course, that we fail just as badly if we give our allegiance only to a passion for abstract knowledge, only to a tolerant but therefore detached appraisal of human convictions, only to the existence of the individual and not to his relationships. The educated life expresses itself above all in a victory over these polar attitudes; it sees that wisdom can come only from the fusion (not just the co-existence) of learning and experience; it sees that responsible society can only be based on the interaction of single life and common purpose; it sees that neither wisdom nor society can long survive unless we give our equal allegiance to our own final convictions and to our respect for the convictions of others. It is the power and the duty of the best education to show that these 'related opposites' are essential to one another if the life of the mind is to flourish, and equally if the life of the community is to be possible.

I can put our responsibility best, perhaps, by saying that though these disparate human qualities need to be brought to a common life in every aspect of our society, it is only the undergraduate college, with all its pedantries and all its social follies, that can take full advantage for this purpose of a discipline of study, an intimate community, and a uniquely formative period in life. Our most singular institutions and traditions—our faculty determination to give all the wrong issues the most heated discussion, our student obsession with freedom and fumbling at responsibility, our trustee rule which combines amateurism so curiously with final control—all these things which are the material of vitriolic novels are really not the base for satire at all, but the base instead of a way of life and a definition of the mind. Without faculty

independence, student freedom, or trustee non-professionalism the need to reconcile opposing attitudes and judgments would never arise, and the real nature of a democratic society would never become integral to the college. We would then be machines devoted to instruction; instead it is our pride that we are living institutions devoted to education.

From the struggle, the diversity of judgment which are the heart of the liberal college we derive, not only the organic nature of the place but also its power to foster creative life and a sense of community. Neither of these central human concerns can exist except in a setting of freedom on one hand, and the desire to make order out of freedom on the other. Mere freedom leads us to chaos; mere order leads us to decay. The life of the mind, and the life of true community move constantly between the two extremes, creating an order, throwing it away in the profound conviction that a better order can be found, recognizing that neither freedom nor order can be allowed to exist without the other. The philosophical and indeed theological base of all valid education consists in this perhaps; a firm enough faith in the final values so that we can endure all kinds of darkness along the way, but a recognition that without the darkness we shall never grow in wisdom or community at all.

One of the most tragic aspects of our 'public culture' at the moment is precisely its fear of this creative darkness. We are conscious enough of the other kinds of darkness, those that lurk always at the edge of foreign policy, stare out of a mental hospital or up from the sidewalks of a slum. The darkness of unknowing, however, makes inner demands on us which we prefer to ignore or to escape; our pride will not accept the dark humility of ignorance, and we forget that it is the only prelude to knowledge. We are willing as a society to repeat the most trivial newsprint version of reality rather than puncture with a question our own pretences at understanding.

In the face of so much bogus knowledge and so little real wisdom it is our place in the liberal colleges to maintain above all that the educated man is the man who will move ahead of his learning, not the man who hides behind it. This man, whom, we hope, we have some share in creating, lives out again and again the central paradox of the Hebrew-Christian tradition. He saves his life by losing it; he risks his security by giving himself to a new idea, an untested theorem, a change of heart. He takes the chance of being wrong, in the faith that the only unforgiven sin is the lack of enough courage to take that chance. Every true community, and every living idea, are the results of just such courage; and it is the central task of the liberal college to foster it, develop it, and make certain that it thrives in our society.

If I am at all accurate in this estimate of the best undergraduate education furthermore, I have also been describing the major responsibility of the Rockefeller Institute. The setting is different, the measurable task is more complex in certain ways; but its twin bases are the creative intellect and the living community. More is expected than the advancement of knowledge, more than the interaction of the great scientific disciplines. When the first graduate Fellow was added to the community of scholars at the Institute, two new responsibilities were assumed: first, for the continuing life of the mind as something shared equally by scholar and pupil; and secondly, for a public image of academic achievement as inseparable from the worthy ends of society at large. The Institute is an instrument of public policy as well as private research; it embodies a way of living as well as a way of knowing.

There have been times in Western society when this way of living (the way of the best undergraduate colleges, and the strongest graduate centres) was not as important as it is to-day, and as it will be in the next half-century. It is a commonplace, but a crucial one, to point out that we are in the midst of a world revolution. Not quite so taken for granted, however, is the fact that this revolution involves every aspect of our internal as well as our external life; and recognized scarcely at all is the fact that only the awareness and conscience of educated men can give us any hope of turning this revolution to creative ends rather than destructive ones.

The truth of the matter, however, confronts us wherever we turn. The tragic recent tangles over national security, for example, almost succeeded in destroying the tradition of responsible dissent. For a year or two many of us were faced with the specious choice between dictated conformity and scorned or discredited independence. We forgot that our political tradition demanded of us loyalty *and* freedom, not loyalty *or* freedom; and in forgetting this we ignored the first mandate of the educated life, that we learn to live with the painful complexity of the truth: and we have made the same error in our social order, of course; we tend to assume far too easily that our social hope and purpose are tied to our progress from group to group; the conflicts which we allow are the thoughtless conflicts of one conformity with another, rather than the thoughtful and productive conflict between individuals and their social order.

Those of us in the academic life are no more exempt from these conformities than our neighbours in industry or government. We have our own clichés, of course, but too commonly we are their victims rather than their masters: and this is true not only if we scrutinize our participation in the social structures of our time but also if we look honestly at our own professional commitments. We assume far too easily that the truth has a departmental structure to it, and as a result we run the constant risk of scattering and dispersing the creative centre of knowledge, which lies primarily not in learned information but in learned interpretation, not in the fragmenting of reality but in its understanding. Here again, learned insight means little until it becomes educated insight, until its real purpose is the mastery of what Yeats described as "the bitter fury of complexity".

The most obvious and insistent demand on educated insight to-day comes, as all of us know, from our emerging international relationships. Here we face in its clearest public form the question which privately animates every true scholar, student, citizen; how I am to keep my identity, my own sense of significance, while I do proper justice to the identity of others? It is this failure as a nation quite to believe in the identity of others that has caused some of our major difficulties in the last fifteen years; and we shall solve them only if we bring to the task of creating foreign policy the most aware and charitable insights of our best-educated minds.

All these areas in which policy and performance must grow creatively are aspects of that central revolution of our time which is a revolution in value. The struggles of a hundred societies to-day are dedi-

cated, not to the simple overthrow of everything that has been believed in the great cultural traditions, but to the radical reassessment of every basic conviction about the nature, purpose, and proper conduct of human life. If we try to pretend that anything less than this is demanded of us by our time, then we have not even begun to act like educated people. We must affirm in the boldest way that the demands of the future are demands above all upon the mind and spirit, and that they can be met only by the deepest efforts of which the educated mind and the learned heart are capable.

THE ROLE OF THE SCHOLAR IN SOCIETY

A SYMPOSIUM under this title was held in Tunis during April 13–18. There were twenty-five invited participants, drawn from fourteen different countries; the study group meeting was organized by the Committee on Science and Freedom, of which the chairman is Prof. Michael Polanyi. The meeting was one of a series of international seminars under the general title "Tradition and Change—Problems of Progress," financed by a special grant from the Ford Foundation to the Congress for Cultural Freedom. This is an international cultural organization with headquarters in Paris, which finances the activities of the Committee on Science and Freedom.

The meeting in Tunis was concerned with the special problems of new (or newly expanded) university institutions in the rapidly developing regions of Africa and Asia, including the Arab countries of the Middle East, and in particular, with the proper relationship between these universities and the society which they serve. Tunisia is a rapidly developing African country which is planning to establish a new university, and a gratifying feature was the interest shown by the Tunisian Government, coupled with the active participation of the Tunisian members of the study group, notably Prof. Ahmed El Fani, of the Free University of Tunis, Mr. Lamine Chabbi, a former Minister of Education, and Mr. Mahjoub Ben Milad, head of the teachers' training college. The chairman of the study group was Dr. J. W. Cook, vice-chancellor of the University of Exeter, and much of the discussion centred on papers which had been prepared in advance by some of the participants.

The Tunisian Minister of Education, Mr. Mahmoud Messadi, presided at the public inaugural session, and in a stimulating and scholarly address referred to Tunisia's cultural background, and outlined the rapid educational progress which has been made since independence, and the plans for future development. Already about a fifth of the national income is being spent on education; nearly half the children now receive primary education, which is expected to become universal within the next ten years. There are several institutions of higher education, including a training college for secondary school teachers, a traditional Muslim university, and the Institut des Hautes Études, affiliated to the University of Paris. Some of these will constitute the nucleus of the new university. In a subsequent session, Mr. Chabbi gave a more detailed and penetrating analysis of the prospective functions and scope of the new university, closely in line with the traditional concepts of a university of Western Europe. He emphasized the need for autonomy, and stressed the importance of vocational guidance with a view to a satisfactory distribution of students among subjects at a time when, for example, the demand for engineers and technicians could absorb the entire output of the secondary schools. He envisaged the university as a lively institution orientated to the service of the community, its research activities being planned in co-operation with consultative bodies representing government and industry.

Prof. B. A. Fletcher, vice-principal of the University College of Rhodesia and Nyasaland, went even further in his concept of the social functions of a new university in a new country, and indicated how the institute of education under his direction provided an advisory service for the government and for industry. This view was challenged by Prof. Polanyi who, while accepting the principle that individual members of a university might be available for consultation, insisted that the university as a body should not attempt to offer authoritative opinions. He remarked that the Royal Society had long since found it expedient to refrain from giving a collective opinion on scientific matters on account of its earlier unfortunate experiences. Prof. Polanyi also disagreed with the suggestion of Prof. Fletcher that the great discoveries of modern physics have been the result of team work. According to Prof. Polanyi, all the great scientific discoveries are individualistic. He was sceptical about the feasibility of control and co-ordination of research, and claimed that research in the natural sciences is self-co-ordinating. Scientific discovery depends on the reliability, the systematic character and the intrinsic interest of the work. He defined the fields of enquiry for which the university is the only place, and those, stimulated perhaps by the possibility of practical application, which may also properly be carried out in the university. Unsystematic technology and invention, he thought, should have no place in the university.

Prof. E. G. Malherbe, vice-chancellor of the University of Natal, referred to the industrial research institutes established in association with his university, the head of the institute being given professorial rank. This helped to interest students in careers in industrial research, and the institutes often encountered long-term theoretical problems suitable for academic study in the appropriate university departments.

There was much discussion about the claims of society on the university in a new country, and how they could be met in ways consistent with academic freedom and university autonomy. Much was said about the pressures liable to be exerted for control by government of the output of teachers, engineers and other professional personnel; of the pressure to increase numbers and to reduce the length of courses in the interests of more rapid output. It was agreed that such pressures should be resisted. But although there seemed such general agreement about these principles in the abstract, one had the impression that different members of the study group interpreted them variously, in the light of the circumstances of their own countries. It is not surprising that the

attitude of the community to the university in a poor and rapidly developing country is inclined to be more utilitarian than in a country where both the community and the university form part of an old established order. However, in the last analysis, as Dr. Salwa Nassar, of the American University of Beirut, so aptly remarked, the role of the scholar in society is to be a scholar.

Prof. Ehsan Naraghi, of the University of Tehran, wondered if pure research was too much of a luxury in an under-developed country. This brought a spirited rejoinder from Prof. Edward Shils of Chicago, who insisted on the need to have in the university the stimulus of taking part in extending the frontiers of knowledge. There was, perhaps, insufficient attention given at the meeting to the criteria for establishing and maintaining academic standards.

In such a gathering at such a place, constantly recurring themes were, not surprisingly, the influence of religion and the problem of language. Dr. Romila Thapar thought that authoritarian religious traditions in India had promoted an attitude in the student of unquestioning acceptance. Mr. M. Bashir was even more gloomy about the stultifying influence in Pakistan of this authoritarian concept of education. As this was linked with the Islamic religion, his views were hotly contested in a meeting where the Arab countries were strongly represented. An interesting point which some had not appreciated was that the problem of the language to be used as the medium of instruction was not the same problem in the Arab countries, as it is in India, for example. In the latter case the choice is between English and a synthetic national language, Hindi, bearing little resemblance to the host of different languages in use in different parts of the country.

In the Arab countries, there is a single national language and the choice is between English or French, and Arabic. In Tunisia the matter had been virtually decided by the decision of the Government to adopt bilingualism (Arabic and French). Mr. Cecil Hourani, adviser to President Bourguiba, made a strong plea for the simplification and clarification of the Arabic language. Attempts to give a complete education in the Arabic language, as is attempted at Damascus, cannot be successful, he thought. Re-fertilization of the Arab mind can take place only by contact with non-Arab sources. This view did not pass unchallenged, and the Arabic language was defended by the Lebanese Ambassador to Morocco, H. E. Selim Haidar, who claimed that Arabic is by its nature a scientific language; he thought that an Arab student would gain just as much from instruction in modern science in Arabic as in a Western language. Interesting views were expressed by Mr. Cecil Hourani, and by his brother, Mr. Albert Hourani of Oxford, about the present and future roles of the three types of university which are found in some countries of the Middle East; namely, the traditional Islamic institutions, the 'foreign' universities, such as the American University at Beirut; and the modern State universities. Only the latter were thought likely to survive as significant influences in the national life.

The idea of a 'foreign' university out of touch with indigenous culture, nationalist sentiment and tribal loyalties was evidently in the minds of the two Nigerian contributors from Ibadan—Dr. E. Njoku, who sent a paper, and Dr. H. A. Oluwasanmi, who attended the meeting. Nigeria demanded a university as good as those in other countries. If academic freedom is a necessary element, then it must exist in Nigeria. But the principle of academic freedom is too shallowly rooted to withstand the over-riding claims of the tribe. Cultural activities, being largely European, make the university seem more exotic than it is, and this causes resentment.

The Turkish participant, Mr. Osman Okyar, suggested that the wave of nationalism which accompanies independence may be a passing phase, and he instanced the support now being given by his Government to university institutions of foreign origin.

The Tunis seminar provided a forum for a useful exchange of views on problems which were approached from several different points of view, and the full publication of its proceedings, which is expected later, will be of interest to all who are concerned with higher education in the emergent countries of Africa and Asia.

J. W. Cook

RADIOACTIVE FALL-OUT

THERE have been four important and authoritative statements on hazards from man-made radiation: a report by the Medical Research Council in Britain, published in 1956[1]; a report by the U.S. Academy of Sciences, also published in 1956[2]; a report by the United Nations Scientific Committee on the effects of atomic radiation, published in August 1958[3]; and a statement on this last report by the Medical Research Council, which appeared simultaneously with it[4]. In a statement which the Prime Minister circulated in the Official Report of the House of Commons (*Hansard*, April 28) he refers to the United Nations report; but his own statements, and in particular those publicized by the Press, are mainly based on the first of these publications, which in some respects has become obsolete.

This is particularly true of the ratio 100 : 22 : 1 (or somewhat more than 1), which plays such a prominent role in the Prime Minister's replies. It is said to be the ratio between the amounts of radiation received (*a*) from natural sources (background radiation), (*b*) from medical and industrial uses, and (*c*) from fall-out due to nuclear bomb-tests. For several reasons this ratio is no longer valid. First, the Medical Research Council has meanwhile revised its estimate of the dose of radiation which the average individual receives through X-radiation. In concordance with the estimate made in 1956 by the U.S. Academy of Sciences, the Medical Research Council in its 1958 statement admits that in countries with highly developed medical services the amount of radiation from diagnostic X-rays may well be as high as the background radiation. In other words, the ratio between the first two items may be 100 : 100 instead of 100 : 22.

For the discussion of radiation hazards from bomb tests this is relevant, because certain radiation effects

are cumulative, and even if X-rays should be used more sparingly in the future, a high amount of X-radiation will have to be reckoned with as an unavoidable ingredient of civilization. It should also be pointed out that, contrary to the Prime Minister's statement, these figures (22 per cent in the older report, 100 per cent in the newer) refer only to diagnostic X-rays, and include neither therapeutic nor industrial radiations.

Secondly, when the Prime Minister states that radiation from nuclear bomb tests amounts to 1–2 per cent of the background radiation, it is not clear to what he refers. At the present time, there is a constant increase in the amount of fall-out, and figures which are valid in one year will be too low already in the next. The Medical Research Council report of 1956 estimated that, if bomb tests were to continue indefinitely at the then existing rate, an equilibrium between formation and decay of radioactive matter would be established in about a hundred years and that in this equilibrium state radiation from fall-out would be approximately 1 per cent of that produced by natural sources. However, a warning was given that this might represent an under-estimate if "present rates of firing were increased and particularly if a greater number of thermonuclear weapons were tested". From the fact that strontium fall-out during the past year has increased far beyond expectation, we may suspect that this warning is applicable, and that the 1956 estimate of fall-out rate at equilibrium was an under-estimate.

Cæsium-137, Carbon-14 and Strontium-90

There is a further reason why the Prime Minister's estimate of man-made radiation is too low. This is the discovery of two more radioactive elements in fall-out, namely, cæsium-137 and carbon-14. The former had just been discovered when the 1956 report of the Medical Research Council came out. It is briefly mentioned in this report, but its effects are not included in the estimate of fall-out. In the United Nations Report this omission has been amended, and the final figure for fall-out radiation, if tests should continue at the present rate, is several times as high as that quoted in 1956 by the Medical Research Council, which accepted this higher figure in its 1958 statement. This means that the amounts of radiation received at equilibrium from natural sources, from diagnostic X-rays (*not* including radiotherapy and the use of radiation in industry and research) and from fall-out are estimated to occur in the proportion of 100 : 100 : between 2 and 4. However, even this is still an under-estimate of the amount received from fall-out; for quite recently—too recently to be mentioned in the United Nations Report—carbon-14 has been found to be present in fall-out from nuclear explosions. According to Prof. L. Pauling, carbon-14 is by far the most dangerous component of fall-out, because it is produced in large amounts at nuclear explosions and takes thousands of years to lose its radioactivity.

It should be stressed that strontium-90 is peculiar in its effects in that it is taken up selectively into the bones, especially those of growing children. The amount so taken up is evidently related to the amount coming down in rainfall, but the relationship is far from straightforward. It depends on the type of soil and vegetation in the fall-out area and on the kind of food by which it is introduced into the human body. The United Nations Committee estimated that for the same amount of fall-out the average concentration of strontium-90 will be five to six times as high in the bones of peoples living mainly on a plant diet than in those of peoples with a milk-type diet. The Medical Research Council accepted this estimate in its statement.

Biological Hazards of Radiation

A clear distinction should be made between effects on the individual and effects on the human race. The former result in sickness and death of exposed individuals; the latter are a threat to the future of mankind.

Effects on the individual. High doses of radiation cause sickness which may result in death; but, barring accidents, nuclear bomb tests will not cause exposures to such high doses. Low doses may result in cancer, often after many years of apparent well-being. The main types of cancer which may be expected are leukæmia and bone cancer, the latter in particular through accumulation of strontium-90 in bones. For animals, there is also convincing evidence that low doses of radiation shorten life-span without obvious disease. Whether this is true also for the human species is still under debate; it certainly is necessary to keep the possibility in mind.

Genetical effects. These occur in the form of gene mutations, and result in hereditary changes. There is no recovery from mutation. Once a mutation has occurred, there is only one way by which mankind can get rid of it: death or sterility of the individuals carrying the mutated gene. Mutations may be good, neutral or harmful in their effects; but in any species which, through long periods of natural selection, has become endowed with a well-working assembly of genes, the vast majority of mutations are harmful. For reasons which cannot be discussed here, even high doses of radiation are not likely to result immediately in the birth of malformed or otherwise obviously handicapped children, except when a pregnant woman is exposed. But any increase in the number of mutations is bound eventually to increase the number of such abnormal children, even though their occurrence may be spread over centuries. In addition, there will be a similar increase in the number of less-drastic hereditary changes, and these will swell the proportion of chronically ailing, emotionally unstable, mentally dull or otherwise sub-standard types in the human race.

The Threshold Concept

There is full consensus among scientists that radiation produces harmful effects on the exposed individual as well as on the human race as a whole; but opinions differ as to the magnitude of the damage expected from nuclear bomb tests. This is not surprising in view of the difficulty of obtaining pertinent data for human beings and the uncertainty factor involved in extrapolation from animal data. Questions directed in the House to the Prime Minister have shown that some Members of Parliament are aware of the key position which the threshold concept holds in all attempts to assess radiation damage quantitatively. Mr. Aneurin Bevan stressed the point that organisms differ in their sensitivity to radiation. This is true, but it is not relevant to the question whether or not there is a lower threshold to certain types of radiation damage. Whether or not

there is such a threshold, individuals, organs and cells may and do differ in their sensitivity to radiation; but where a threshold exists, doses below a certain level of exposure—which may not be exactly the same for every individual, organ or cell—are entirely ineffective. Given the right amount of information, one can therefore determine a safe level of exposure below which no damage is to be expected. Where there is no threshold, no such safe level exists, and even the lowest exposure will produce damage in proportion to its magnitude.

There is very good evidence for affirming that there is no lower threshold for the production of mutations by radiation. In view of misleading articles in the daily Press, I want to emphasize that this conclusion is not invalidated by very recent experiments on mice, which indicate that the genetical effectiveness of radiation in certain types of cell depends on the time over which a given dose of radiation is spread. Estimates of the actual amount of genetical damage involve so many assumptions that they cannot be more than very rough approximations. For the case that bomb testing continues at the present rate, Prof. Pauling estimates that in about a hundred years, when equilibrium has been reached, about 15,000 seriously defective children will be born each year as the result of the tests. This value is within the wider limits of 1,000–40,000 adopted by the United Nations Committee. It does not include abortions, miscarriages, stillbirths and many minor hereditary defects which will have been produced at the same time. Nor does it take account of carbon-14 which, because of its very slow rate of decay, continues to produce radiation damage over many thousands of years. According to Pauling, already the bomb tests carried out so far "will ultimately produce about 1 million seriously defective children and about 2 million embryonic and neonatal deaths, and will cause many millions of people to suffer from minor hereditary defects".

There is as yet no evidence available which would allow a decision as to whether or not there is a lower threshold to the production of cancer, leukæmia and, perhaps, premature ageing by radiation. Recognizing this, the United Nations Committee has made all its calculations concerning these effects in duplicate: once on the assumption that a threshold exists, and a second time on the assumption that it does not exist. In the first case, as in that of mutation, even the lowest additional amount of radiation is dangerous in proportion to its magnitude; in the second, it seems likely that the present amount of fall-out is still below the danger level.

It should, however, be evident that in a matter like this—a matter of life and death for hundreds of thousands—one should act on the basis of the more pessimistic view until convincing evidence for the more optimistic one has been furnished. This seems also the opinion of the Medical Research Council. In its 1958 statement it writes: "In our view, it is not possible at this present time to decide whether there is or is not a threshold dose concerned in the induction of leukæmia and cancer, and the only scientific attitude to the problem at present is one of suspended judgment. Nevertheless, the significance of the alternative points of view in determining the ultimate assessment of risk should be clearly understood so that those who have the responsibility for acting on such assessments should be fully aware of the alternative possibilities that need to be taken into account".

The Role of Strontium

Strontium, as has been pointed out, plays a special part as a potential cause of bone cancer. It is therefore very disturbing to learn that the amount of this element in the rainfall over the United Kingdom has doubled during the past year. It is difficult to understand why this seems to cause little anxiety to the Prime Minister and his advisers. The amount of strontium-90 in the bones is measured in strontium units (1 strontium unit (S.U.) = 1 micromicrocurie of strontium-90 per gram of calcium). 1,000 S.U. is the industrially permissible dose; but in 1956 the Medical Research Council recommended that in a population with many growing children the allowable dose should not exceed an average of 100 S.U. and "that immediate consideration would be required if the level in the general population showed signs of rising greatly above 10 S.U.". The United Nations Report of 1958 presents figures which show that at the end of 1957 the average bone content of strontium-90 was 2·3 S.U. in countries with a milk-type diet and 14 S.U. in plant-eating communities, in both cases as the average over the next 70 years. This was before the spectacular increase of strontium-90 in rainfall, and although—as pointed out above—the relation between strontium in rainfall and strontium in the bones is complex, it cannot be doubted that this increase in fall-out will be reflected in a marked increase in the strontium-90 content of bones. If there has been a similar increase of strontium-90 fall-out in other parts of the world, the strontium content of the bones in some plant-eating peoples may already be well in excess of 10 S.U.

In regard to the future, the United Nations Committee estimated in 1958 that, if bomb tests continue at the same rate as between 1954 and 1958, the content of strontium-90 in the bones of people living about a hundred years hence will be 40 S.U. for countries with a milk-type diet and 240 for countries with a plant diet. Such figures, according to the Medical Research Council report, would certainly call for immediate consideration. Since the recent rise in strontium-90 fall-out has been so unexpectedly high, this situation may arise in a much shorter time.

Background Radiation

The Prime Minister seems to think it a cause for complacency that the human race has survived in spite of a considerable amount of radiation from natural sources. The opposite is true. It is highly probable that natural radiation causes many cases of leukæmia and bone cancer. There is nothing we can do about it. We just have to put up with it, as we have to put up with earthquakes and hurricanes. But the fact that many people are killed through such natural catastrophes does not make us complacent about death-rates from industrial accidents or careless driving. The fact that there is this inescapable amount of natural radiation, and that recently there has been added to it probably more than an equal amount of medical radiation, makes it even more imperative to keep radiation from any additional sources as low as possible.

For the genetical effects of radiation the situation is somewhat more complex and cannot be discussed here in detail. Only so much can be stated: mutations are the ultimate raw material for natural selection. Without mutation, there would have been no evolution. Naturally occurring mutations, too, are usually harmful, but Nature—in contrast to

man—can afford to waste thousands of ill-adapted individuals for the sake of a single better adapted one. It is probable that in the course of evolution every species establishes a balance between the frequency with which its genes mutate and the amount of natural selection to which it is exposed. It is also probable that for mankind this balance has already been upset through the relaxation of natural selection, and that the frequency of even natural mutation may be rather higher than is desirable. Any additional mutations will shift the balance even further in the direction of genetical deterioration.

Manufacture of Nuclear Devices by Private Firms

I should like to make one final point. Prior to the discussion of radioactivity in the House of Commons on April 28, the Prime Minister was asked a question regarding safety measures in private firms which produce nuclear devices. The Prime Minister answered that the Atomic Energy Authority exercized the same sort of control and supervision in these firms as in its own establishments doing similar work. No doubt this is true; but there is a different angle to this question. The industrially permissible dose has been lowered repeatedly, and at present is only 1/14 of what it was in 1934. Yet it is still fairly high, and, if received in full by many employees for many years, would be a cause for concern, especially to geneticists. It is, however, asserted that very few workers in Government plants receive anything like this dose. In 1957, Prof. H. J. Muller, the leading expert on the genetical effects of radiation, pointed out that in private firms "the pressure of costs will tend to prevent the observance of more rigorous standards than those officially recognized, and there is therefore a danger of considerable radiation being received from the so-called peaceful atom, unless we further lower the ceiling". It would seem that in the United Kingdom we are reaching the stage where such a lowering of the officially permissible dose for employees in nuclear establishments should be taken into serious consideration.

C. Auerbach

[1] Medical Research Council. The Hazards to Man of Nuclear and Allied Radiations. (Cmnd. 9780.) Pp. vii+128. (London: H.M. Stationery Office, 1956.) 5s. 6d. net. See *Nature*, **178**, 453 (1956).

[2] National Academy of Sciences–National Research Council. The Biological Effects of Atomic Radiation: a Report to the Public from a Study by the National Academy of Sciences. Pp. ii+40. The Biological Effects of Radiation: Summary Reports from a Study by the National Academy of Sciences. Pp. xiv+108. (Washington, D.C.: National Academy of Sciences–National Research Council, 1956.)

[3] Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. (General Assembly—Official Records: Thirteenth Session, Supplement No. 17 (A/3838).) Pp. iii+228. (New York: United Nations; London: H.M. Stationery Office, 1958.) 2.50 dollars; 18s.; 10.50 Swiss francs. See *Nature*, **182**, 1543 (1958).

[4] Medical Research Council. Statement on the Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. Pp. 15. (Cmnd. 508.) (London: H.M. Stationery Office, 1958.) 1s. net. See *Nature*, **182**, 1543 (1958).

OBITUARIES

Dr. Jesse P. Greenstein

Biochemistry and cancer research lost one of its leading figures with the sudden death on February 12 of Jesse Phillip Greenstein. He had been chief of the Laboratory of Biochemistry at the National Cancer Institute, Bethesda, Maryland, since 1946 and held this position at his death.

Born in New York in 1902, he graduated first from the Polytechnic Institute of Brooklyn in 1926 and then took an M.S. and finally Ph.D. in biochemistry at Brown University. In the next two years he held fellowships at Harvard (Prof. E. J. Cohn) and at the Kaiser-Wilhelm Institute, Dresden (Prof. M. Bergmann). This one year spent with Bergmann in Germany confirmed Greenstein's interest in amino-acids and settled much of the pattern of his future researches. Two valued reference books he always kept to hand on his desk were the collected works of Emil Fischer and of Max Bergmann. He returned to Harvard and during 1933–39 was instructor in biochemistry at Harvard College, and a research associate of Harvard Medical School. His research interests in this period were concerned with peptide synthesis, the physico-chemical characteristics of peptides in solution and the role of sulphydryl groups in protein structure and denaturation. Much of this work was of a new and fundamental nature and has retained a permanent place in the literature.

Leaving this special field of research, Greenstein entered the second and major phase of his career when in 1939 he moved to join the staff at the National Cancer Institute. He extended his earlier interests to include nucleoproteins and nucleic acids, and then with a team of workers devoted his energies to a survey of the enzyme spectra of living cells, normal and neoplastic. Greenstein's activities in this field undoubtedly stimulated the post-war surge of research into the chemistry of growth. With his colleagues he published more than a hundred papers in the period 1940–47 dealing with this problem. Much of this work was incorporated in his first book published in 1947, "Biochemistry of Cancer". This book has remained the most important and most quoted survey of experimental cancer research. It was revised and of necessity greatly extended in its second edition in 1954 and is unlikely to decrease in value for many years to come.

While retaining an interest in enzymology, Greenstein turned his interest back to amino-acid chemistry. Papers published over the period 1948–58 describe the use of enzymatic methods for the resolution of amino-acids. Making use particularly of a kidney enzyme, designated 'acylase', Greenstein and his colleagues showed that by asymmetric hydrolysis of acyl-DL-amino acids both the pure L- and D-forms could be obtained in good yield and of high optical purity. During the past three years the application of these pure isomers in nutritional and metabolic experiments was being studied.

The volume of published work conveys some idea of Greenstein's immense energy. The carpeted office approached through a protective screen of secretaries was not for him. Instead, there was an open door and a pair of hands ready, indeed anxious, to help with practical work and a fund of advice and experience. He was always anxious to help along the younger workers of his group and would always give them full credit for any achievements. He also afforded a very generous welcome to workers from many countries, and the hospitality of his family, as well as of the laboratories, must have left them with a very happy impression of American life.

Greenstein himself went outside the United States on two occasions only—to a conference at the Papal Academy in Vatican City in 1949, and to Japan in 1956. He did, however, take a very prominent part in American scientific affairs. He was, for example, a past chairman of the Division of Biological Chemistry of the American Chemical Society and a member of the National Research Council's Committee on Biochemistry. He was on the editorial boards of *Cancer Research* and *Archives of Biochemistry and Biophysics*, while in recent years he was joint-editor with Prof. A. Haddow of "Advances in Cancer Research". At the time of his death he was just completing a major treatise on amino-acid chemistry. Among many formal acknowledgments of his work were included a Distinguished Service Award from the U.S. Government (1954) and the Hillebrand Prize of the American Chemical Society (1957).

Though modest about his own contribution, he was visibly proud of the work of his section, of his country and above all of his family. He will be greatly missed and all will sympathize with those near to him at their loss.

DOUGLAS HAMER

DR. GREENSTEIN'S contributions extended widely in the fields of amino-acid, peptide and protein chemistry, and of nutrition. In the past few years he had devoted much effort to the elaboration of a completely soluble diet composed of chemically defined constituents; and at the time of his death he was engaged (with M. Winitz), in the final stages of a major work on the chemistry of the amino-acids, which is to appear in three volumes, of which the first is now in the press. But apart from these, his contributions to cancer research alone would have more than sufficed to establish him among the leading biochemists of his time. He was an accomplished writer, and his "Biochemistry of Cancer" had, and has, immense influence, and proved a very significant stimulus to the subject as a whole.

Of his own work, two aspects were of particular importance; first, his studies of the mechanism of production—and the significance—of the dramatic decline in liver catalase activity which is observed in tumour-bearing animals; and secondly, his analysis and comparison of the distribution and activity of a host of enzyme systems in normal and cancer cells. In spite of the extreme difficulty and complexity of these problems, there gradually emerged a real and satisfying conclusion, namely, that although tumours mostly possess the same enzymes as do normal tissues, qualitatively, none the less they tend, enzymatically, to converge to a common type, and hence show more resemblance to one another biochemically than to the normal tissues from which they arose.

All these, and many other contributions, embodied in upwards of 250 papers, brought him international acclaim. But we shall also remember him for his human qualities, and his combination of serious devotion with much courage and humour. Certainly I have good cause to be grateful for many years of happy association with him; and Bethesda without him can never be quite the same. All who admired him will be glad to know that there has now been established, in his remembrance, a Jesse P. Greenstein Memorial Foundation, to be administered by a small committee of his colleagues, and which will, in cases where it is necessary or helpful, provide tuition and support during the years of undergraduate study in the arts or sciences, "to the son or daughter of a scientist who has made notable contributions to the medical, physical or biological sciences, and who is deceased".

ALEX. HADDOW

Dr. Arthur Walton

DR. ARTHUR WALTON, who died suddenly in Cambridge on April 6, was best known for his pioneering research in the field of sperm physiology and artificial insemination of farm animals. He was born in London, on March 16, 1897, of Scottish parents, and educated in Edinburgh, first at Daniel Stewart's College and later at the University, where he obtained in 1923 the degree of bachelor of science (agriculture). It was also in Edinburgh, at the Animal Breeding Research Department, where he received his early training in research methods and published, in 1924, his first paper, on "The Flocculation of Sperm Suspensions in Relation to Surface Charge". Keen interest in sperm physiology prompted Walton to move to Cambridge and take up research on animal reproduction. In 1926 appeared his first communication in *Nature*, on "Preservation of Mammalian Spermatozoa", a subject which he expanded in the following year in his Ph.D. dissertation, and to which he devoted his special attention throughout his scientific life.

Among Walton's most outstanding contributions to the subject of sperm physiology was the first demonstration that ram semen, properly collected and stored, can be sent on long journeys to distant countries, to be used there for successful insemination of ewes; next, there were the experiments which proved that 'cold shock' can destroy the fertilizing ability of bull and ram spermatozoa. Of great scientific significance was his observation that the metabolic activity of ram and bull spermatozoa, particularly their respiration, is directly correlated with motility; and the discovery that respiring spermatozoa produce under certain conditions hydrogen peroxide which, in turn, is responsible for a gradually developing inhibitory effect on respiration and a decline in sperm motility. During his more recent investigations, Walton developed a novel and ingenious method of maintaining sperm alive for long periods, in a perfusion apparatus where nutrient substrates are fed continuously to a sample of semen and the toxic metabolites removed at the same time.

In addition to the work on spermatozoa, Walton has been keenly interested in various other aspects of reproductive physiology, including mating behaviour in animals and female fertility. He was the first to make detailed observations on ovulation in the rabbit, and to study the effect of maternal influences on the size of offspring in reciprocal crosses between large and small breeds of horses.

As a member of the University School of Agriculture and the scientific staff of the Agricultural Research Council, Walton spent nearly thirty years of his life in Cambridge, much of it in his laboratory at the Animal Research Station, in close association with many young research workers, always ready to help, to guide, and to advise. Although primarily concerned with physiological problems, Walton had also a profound knowledge in the field of general biology and agriculture. His contributions to agriculture, especially those which led to the establishment of the practice of artificial insemination in farm animals, were recognized in 1957, by the award of

the Medal "for outstanding research in agriculture" from the Royal Agricultural Society of England. Walton's many friends all over the world will mourn the untimely death of this outstanding scientific worker.

J. Hammond
T. Mann

Dr. F. Tattersfield, O.B.E.

Dr. Frederick Tattersfield died at his home in Harpenden on May 1 at the age of seventy-eight. He may well be described as the founder of modern research on insecticides.

Dr. Tattersfield was born near Dewsbury in Yorkshire and went to Dewsbury Grammar School and the University of Leeds. From Leeds he graduated in chemistry with first-class honours. His first job was in association with the Leeds City Analyst, where for a number of years his work ranged over all the typical activities in such a department. In 1908 he joined a paint manufacturing firm; here his work was chiefly concerned with research on anti-fouling paints for ships.

Dr. Tattersfield went to France in the First World War as a founder member of the Friends' Ambulance Unit. He was invalided out in 1917, and went to Rothamsted Experimental Station, where he remained for the rest of his working life. He originally had a temporary appointment in the Chemistry Department, but he soon founded the present Department of Insecticides and Fungicides, of which he was the head for twenty-nine years.

Dr. Tattersfield's earliest work was concerned with the control of soil pests and he carried out some work on the structure – toxicity relationships of chemicals to wireworms. He also studied the factors influencing the decomposition of naphthalene in the soil and the effect of different rates of decomposition on its insecticidal action. He then proceeded to study the effect of a range of chemicals on the insects that attack the aerial parts of the plant, again attempting, so far as possible, to relate toxicity with structure in some systematic way. In the course of this work he discovered the outstanding ovicidal properties of dinitro-*o*-cresol, a substance which has been used as a winter wash for fruit trees ever since. At that time chemical manufacturers had little confidence in the development of effective synthetic organic chemicals for pest control, and Dr. Tattersfield in his search for highly biologically active chemicals turned his attention to plants. He examined a wide variety of plants for insecticidal activity, but his main work was done on the fish poison group, particularly *Derris* spp. and *Tephrosia* spp. and on *Pyrethrum*. His contributions on the isolation of the active principles of these plant products, the assessment of their insecticidal activity and their chemical estimation were quite outstanding. He studied many phases of the production and assay of pyrethrum as an insecticide and played a large part in the founding of the Kenya pyrethrum industry, an industry which has proved of the greatest value to the economy of the country.

During the course of his work, Dr. Tattersfield evolved precise methods of administering doses of chemicals to insects and introduced statistical procedures for the quantitative assessment of results.

The mere cataloguing of Dr. Tattersfield's contributions to knowledge on insecticides, substantial though they are, gives a very inadequate idea of what the subject owes to his influence which, fortunately, was widely felt owing to his high international reputation. When he entered the subject the standard of work was very low and no serious attempt was being made to obtain reproducible quantitative results of known significance. Dr. Tattersfield insisted on the importance of precise quantitative data where the factors known to influence the results were standardized, so far as possible, and where both the design of the experiment and the results would satisfy accepted statistical criteria. In doing this he set standards which, over the course of years, have been accepted to the inestimable benefit of the subject.

Dr. Tattersfield was always a source of inspiration to his colleagues, to whom he was unfailingly kind and helpful. His justly acquired high reputation never changed his modest and unassuming personality, and his death will leave many of us with a deep sense of personal loss. He leaves a widow and one son.

C. Potter

Dr. G. K. Groetzinger

Dr. Gerhart K. Groetzinger, a principal scientist at the RIAS Division of the Martin Company, Baltimore, died on March 30 at the age of fifty-one. Born in Vienna, Austria, he received his doctorate in physics from the University of Vienna in 1931 and was a member of the faculty there before going to the United States in 1938.

Dr. Groetzinger has been associated as a faculty member, research worker and consultant with the University of Illinois, Ohio State University, the Enrico Fermi Institute for Nuclear Studies at the University of Chicago, the Lewis Laboratory of the National Advisory Committee on Aeronautics, and the Los Alamos Laboratory of the Atomic Energy Commission. Dr. Groetzinger joined RIAS in January 1956, and his work there since then had dealt primarily with solid state physics and cosmic-ray experiments performed in Earth satellites and high-altitude balloons. His work included some of the pioneer investigations of such fields as magneto-photoelectric effects in semi-conductors and the nuclear interactions of mu mesons.

Mr. H. Curtis

Mr. H. Curtis, production director of Leda Chemicals, Ltd., died on May 20 at the early age of thirty-nine.

Mr. Curtis graduated with honours at the Manchester College of Technology in 1941 in chemical engineering. After working during the War with chemical firms, he went to Sondes Place Research Institute in 1948 and in 1950 to Leda Chemicals, Ltd. He was appointed as a chemical engineer at a time when the firm was starting to manufacture fine chemicals. During his service with the firm, Mr. Curtis designed and constructed plant for the manufacture of quaternary ammonium compounds, cyanamide monomer and a range of other fine chemicals, notably rubber chemicals such as alkyl dithiocarbamates.

During the year 1953–54 Mr. Curtis went to Israel and worked on process development with Israel Mining Industries, Ltd. On returning he was elected a director of Leda Chemicals and its associated companies, Cunningham Smith, Ltd., and Lea Valley Chemicals, Ltd., becoming responsible for production and chemical engineering in these companies.

NEWS and VIEWS

Mechanical Engineering at Belfast: Prof. F. V. Warnock, O.B.E.

Prof. Frederick Victor Warnock will retire on September 30 from the chair of mechanical engineering at The Queen's University of Belfast. He received his engineering education at the Royal College of Science, Dublin, and has been a member of the Faculty of Applied Science and Technology at Belfast since its inception in 1921. He is now the sole surviving founder member. He was the first occupant of the chair of mechanical engineering, founded in 1955, though he had served as head of the Department in the Belfast College of Technology and as extra-mural professor in the University since 1938. Many of his former students hold positions of responsibility in universities, industry and research organizations, and they would all testify to his skill as a teacher. A wider public is familiar with his clarity of exposition through his book on "Strength of Materials".

In his long academic career at The Queen's University, Prof. Warnock has been actively concerned in the most successful collaboration between the University and the College of Technology, and as professor and dean of the Faculty, has been responsible for the planning of the new engineering building which will serve the needs of both bodies for advanced engineering studies. His main research interest lay in the shock properties of metals and, though he published a number of papers in the *Proceedings* of the Institution of Mechanical Engineers and of the Iron and Steel Institute, these do not reveal the full importance of his work. During the Second World War he was responsible for some experiments in shock testing of steels on behalf of the Admiralty, and these results have never been published. Much important work has been done on repeated impact testing in other universities by men who learned their techniques and received their inspiration from Prof. Warnock.

The Editors regret the delay in publishing this issue of "Nature". From now on, every effort will be made to bring out succeeding issues as quickly as possible until the normal date of publication is resumed

Dr. B. Crossland

The appointment of Dr. B. Crossland to the chair of mechanical engineering at The Queen's University, Belfast, in succession to Prof. Warnock, has recently been announced. Dr. Crossland was educated at Simon Langton's Grammar School, Canterbury, and Nottingham University College, where he took a London honours degree in 1943. He obtained his practical experience with Rolls-Royce, Ltd., Derby, first as an apprentice and then as technical assistant in the experimental vibrations section which dealt with both reciprocating and the then novel gas turbine engines. After a brief period as a lecturer at Luton Technical College he was appointed to the staff of the Mechanical Engineering Department, University of Bristol, where in 1957 he was promoted to senior lecturer.

In 1947 Dr. Crossland obtained the degree of M.Sc. for a thesis on the "Axial Vibration of Crankshafts" and in 1954 the Ph.D. degree for a thesis on "The Effect of Fluid Pressure on the Shear Properties of Materials". These theses reflect a gradually changing preoccupation from problems in the field of vibrations and machines to those concerned with strength of materials, in particular plasticity and fatigue. To these latter subjects he has, in recent years, made important contributions; for example, in elucidating the effect of hydrostatic pressure on brittle materials, in determining the behaviour of thick tubes subjected to internal pressure, and in investigating the behaviour in relation to fatigue of materials subjected to cyclically varying triaxial stresses.

Automatic Programming of Digital Computers

The first national conference devoted to problems of automatic programming of digital computers was held during April 1–3 at Brighton Technical College. Organized by the Mathematics Department, the conference was attended by 111 representatives of computer manufacturers, research establishments, industry, universities and technical colleges. The conference recommended that Brighton Technical College be asked to establish, with the help of the profits of the conference and the assistance of computer manufacturers, a national information centre on auto-programming. The aim of this centre would be to collect a comprehensive library of documents on the subject, to make available to subscribers, by a postal service and otherwise, copies of any such document required, and to publish, from time to time, in the British Computer Society's *Bulletin*, lists of new documents acquired. It was also suggested that the British Computer Society be asked to co-opt on to its sub-committee concerned with problems of a universal autocode a representative of the Brighton Technical College, as organizers of the conference. A list of definitions of commonly used terms, such as 'interpretive routine', 'translation routine', 'compiler', 'generator', etc., suggested in a paper by Dr. Stanley Gill, was circulated to the participants for comment. This list is also to be published in the Computer Society's *Bulletin* so that any member of the Society who was not at the conference may take part. The proceedings of the conference are to be published by the Pergamon Press.

British Association of Corrosion Engineers

The British Association of Corrosion Engineers was formed at a luncheon given under the auspices of *The Corrosion Engineer* on May 29. The objects of the Association will be generally to promote the dissemination of technical information about corrosion matters and to develop by means of social activities the free interchange of information among

members. In due course the Association, which is, of course, essentially non-profit making, will progress towards the establishment and acceptance of suitable qualifications for corrosion engineers, and the promotion of standardization in the terminology and techniques of corrosion control. When the Association is in full operation it is intended to hold full-scale meetings with papers, films and discussions, which should be of great benefit to the corrosion engineering profession as a whole and which will promote and foster its growth and development as well as being of general service to industry. Further information can be obtained from the Hon. Secretary, British Association of Corrosion Engineers, 97 Old Brompton Road, London, S.W.7.

The Average Length of Working Life

Figures showing the average expectation of working life for males in Great Britain, which have been prepared by the Ministry of Labour and National Service, are set out in a booklet called "The Length of Working Life of Males in Great Britain" (pp. 24. London: H.M. Stationery Office, 1959. 3*s*. 6*d*. net). The publication contains tables and charts which are designed to provide answers to questions like: How many years on the average can men of a given age expect to continue at work? What proportion of the men of a given age in the working population can expect to be still working at sixty-five and then have a period of retirement? What were the estimated losses from the working population in 1955 owing to death and retirements? For example, the 1955 table shows that on the average boys aged fifteen can expect to have 48·4 years in the working population. Of the men aged thirty in the working population, 62 per cent can expect to be still in the working population at age sixty-five. For those men aged sixty-five in the working population, 75 per cent can expect to retire at some later age. A very large population (31 per cent) can expect to retire before reaching their sixty-sixth birthday. A comparison between 1931 and 1955 shows that over this period the expectation of working life at birth rose from 40·6 years—an increase of 12¼ per cent. This was entirely due to the reduction in mortality-rates, which outweighed the effect of the raising of the school-leaving age and of the movement to earlier retirement. There is a growing demand for the kind of information given in these tables because private pension schemes and the new social security legislation introduced into Britain during the present century have made it possible for an increasing number of people to spend a significant number of years in retirement. 'Probability of survival' tables have been in use for a long time, but since there is now a substantial difference at particular years of age between the average expectation of life and the average expectation of remaining in the working population, separate tables show the expectation of working life.

Training for Skill

"Training for Skill", by Lady Gertrude Williams, issued as No. 205 in the Fabian Research Series (pp. 28. London: Fabian Society, 1959. 2*s*.), is based on the factual information regarding boy apprentices collected in a survey, "Recruitment to Skilled Trades" (see *Nature*, 181, 373; 1958). It also covers the Carr report, "Training for Skill", which is criticized for failing to face the profound changes in industrial structure and in public education which have made many of the traditional methods inappropriate to present needs, although the report contains an agreed criticism of the inflexibility of the present regulations. The pamphlet urges that some new method must be found which ensures that an adequate supply of training vacancies is available to provide enough skilled workers for future needs, and that the costs of training are spread more justly over all the firms that benefit from the employment of adult skilled men. It should also ensure that men capable of learning to do a job are not permanently excluded simply because they missed the opportunity to train when they were sixteen years old, that the quality of teaching in the workshops is improved, and that the theoretical instruction is graded to suit the capacities of the pupil. Lady Williams refers to some schemes for group training recently instituted and an extension of the 'sandwich system' as possibilities to be explored, and also suggests that apprenticeship might come to be regarded more definitely as a function of education rather than of industry. In an introduction in which she discusses industry's present needs and the limits of mass production, Lady Williams stresses the real danger that there will not be sufficient skilled workers available to understand and execute the instructions of the increased number of scientific advisers and technologists in industry.

Technics and Purpose

Two additional booklets have been announced in the admirable series launched to examine the issues arising out of the growing importance of scientific techniques in society, the rapid expansion of technological and other education and the purposes and values involved in the light of the Christian faith. The first (On Being a Technologist. Technics and Purpose, No. 5. Pp. 33. S.C.M. Press, Ltd., London, 1959. 2*s*. 6*d*. net), by Prof. D. G. Christopherson (Imperial College of Science and Technology, London), suggests that, contrary to all the pessimistic views of the persuasive advocates of 'Admass', the total responsibility shared by the members of an industrial society has increased. Once this belief is accepted it calls for a large extension of the attitude of mind formerly associated with the professions; many more people will have to make judgments based on knowledge and reason. Such a society will become increasingly individual and less amenable to the techniques of mass suggestions.

The second (The Educated Man Today and Tomorrow. Technics and Purpose, No. 6. Pp. 26. S.C.M. Press, Ltd., London, 1959. 2*s*. 6*d*. net), by Sir John Wolfenden, is noteworthy not only because it paints a clear picture of an educated man but also because it does so without becoming trite, or condescending. Besides those qualities of character which are the hallmarks of education, Sir John believes that the educated man will accept his place in the world of Nature and will see that he understands what the scientists and technologists are doing, the nature of their thinking, and, as their numbers and influence are continually increasing, the way in which they should be educated. The third requisite for an educated man put forward by Sir John shows a remarkable likeness to that advocated by Prof. Christopherson, albeit arrived at from a different approach. Prof. Christopherson writes, "Somehow or other, if a man is to do a proper job he must find means of continuing the process of education through his whole professional

life, and the need to do so is in itself a stimulant to thought not only about the immediate problems of the profession but about the deeper problems of purpose, proportion and responsibility". For Wolfenden, "the restlessness of the human mind is a divine restlessness; and however solid these brick walls may seem to be, it is part of man's nature that he cannot resist banging his head against them. For it is not in the problems of our relations with other human beings or of our relations with the world of Nature that the ultimate problem lies; it lies in our relations with what is neither of these. What is very clear is that an education with this basis will not end at school-leaving age, or with the acquisition of a university degree. There is no reason to suppose that it ends with the grave".

Electrotechnology in Australia

THE annual report for 1957–58 of the Division of Electrotechnology of the Commonwealth Scientific and Industrial Research Organization of Australia describes the three main activities of the Division: the maintenance of the Commonwealth standards of measurement of electrical quantities, including the standard of frequency; calibration of electrical and magnetic instruments; and research on the improvement and extension of the electrical measuring and standards facilities and on the dielectric properties of insulating materials and the microwave spectra of gases. New apparatus installed includes a small vacuum evaporation plant; a calculable capacitor and associated measuring equipment; and facilities for making impedance and other measurements at microwave frequencies chiefly within the 9,000 Mc./s. range. The hyperfine structure of the microwave spectra of heavy-water molecules is being analysed on the high-speed digital computer *Silliac* to determine more accurately the electric field gradient at the position of the deuteron nuclei in these molecules and to estimate the magnetic constants governing the smaller effects observed in the spectra. Special furnaces have been constructed to assist in the determination of the conditions required for the successful growth of large single crystals of organic materials, and the study of the dielectric properties of polycrystalline materials and liquids has been extended. Both experimental and theoretical work has been carried out on dielectric breakdown in the alkali halides and a theoretical study based on the harmonic motion of the interacting electron–lattice system has been made of the superconducting specific heat and transition temperature of multivalent metals.

Wool Industries Research Association

THE report of Dr. A. B. P. Cassie, director of research of the Wool Industries Research Association, presented to the annual general meeting of the Association on April 7, includes, besides details of the membership of the Council and Committees and a list of staff, a bibliography of publications in 1958, including lectures given by members of the staff, patents and some forthcoming publications (Wool Industries Research Association, Report of the Director of Research for 1958. Pp. 35. (Publication No. 212.) Headingley, Leeds: 1959). Modifications have been made to the pilot scouring plant, while methods for determining oil and grease in scoured wool and tops have been extensively investigated. Further work on the pulling of rags has confirmed that there is little, if any, difference in the quality of the yarns or fabrics prepared from the differently treated yarns. The work on examining the state of a yarn at the instant of break during spinning is now almost complete, and a technique has been evolved for determining whether the fibres slip or break when the yarn breaks for use with 'Fibro' but not for wool. Further experience with wool/'Terylene' blends indicates that there can be no guarantee against the tight ends or 'fiddle-string' fault developing, as procedures which are entirely satisfactory with all-wool yarns can give rise to snatch and stretching of wool/'Terylene' yarns without breakdown, but a procedure which seems to provide reasonable protection during cone-winding is indicated. The review of milling was expanded and work continued on the scouring of wool/'Terylene' worsted cloths. The study of damage to wool in dyeing was extended to coarse wool, and work was restarted on the efficacy of dieldrin in moth-proofing. The first part of the work on the examination of the washing process of wool by microscopical methods was completed, and determinations of the latent heat of sublimation of nitrogen-substituted amides, which contain a peptide group similar to that in keratin, suggest that the strength of this bond is 4–5 kcal. Homogenous membranes are now being used to study the effects of organic solvents on the rate of diffusion of dyes into keratin.

East Africa High Commission: Report for 1958

APPROXIMATELY half the illustrated and very readable annual report for 1958, of the East Africa High Commission (pp. iv + 92. Nairobi: Government Printer, 1949. 5*s*.) is concerned with research and scientific services, including some material to be found in "Colonial Research, 1957–58" and in the annual report of the East African Industrial Research Board as well as in "The Colonial Territories, 1957–58". Although more limited territorially, it has a somewhat more popular appeal and is well designed to show the important contribution which scientific research is making to development and welfare in East Africa. This is particularly true of the work of the East African Agriculture and Forestry Research Organization, the Veterinary Research Organization, the Fishery Research Organization, the Marine Fisheries Research Organization, the Trypanosomiasis Research Organization, the Virus Research Institute and the Institute for Medical Research. Expenditure on research was £806,721 and on the Desert Locust Survey £438,982, but it is stated that for the past two years difficulty in recruiting suitable technical and scientific staff has been the main hindrance to the expansion programme.

Acta Biologica Cracoviensia

A NEW botanical journal, *Acta Biologica Cracoviensia* (Sér: Botanique) (1, No. 1; 1958), has been published for the Polish Academy of Sciences under the editorship of Mme. Prof. M. Skalinska (Cracovie. Inst. d'Anatomie et de Cytologie des Plantes; Rue Jana 20). The contents of this first issue are partly palæobotanical, partly cytological. An article on the interglacial flora in Ganovce travertines in Eastern Slovakia is contributed by V. Kneblova; and there are short descriptive accounts of the genus *Cunninghamia* in the European Miocene by W. Szafer and of *Salvinia* and *Azolla* from the same geological horizon in Poland by M. Lancucka-Srodoniowa. The several cytological investigations, by Prof. Skalinska and her colleagues or pupils, deal with the curious behaviour

of nuclei in the anther tapetal layer in species of *Cucurbita*, *Aconitum* and *Valeriana*. Up to a certain point the tapetum consists of normal uninucleate cells, but about the time of the prophase of meiotic divisions in the pollen-mother-cells some unusual cytological developments are induced: while some of the tapetal cells and their nuclei remain as before, others may become polynucleate, and yet others may show more or less conspicuous enlargement and, by endomitosis, may give rise to nuclei of different size categories corresponding to $4n$, $8n$, and even $16n$ chromosomes complements. These developments, described by these authors as "the differentiation of the tapetal cells", are considered to result from successive stimuli which can be related to developmental changes in the developing pollen-mother-cells and the tetrads to which they give rise. On the evidence to date, based on species drawn from widely separated families, it appears that these seemingly anomalous developments in the tapetum may well prove to be of very general occurrence. The texts are in English throughout, with bilingual titles. The journal is well produced, with a good clear type, and is illustrated by line drawings and by photographic plates of good quality.

Link between the Annelids and the Arthropods ?

A newly found fossil from near the base of the Cambrian of South Australia shows affinities which may throw some light on the origin of the arthropods. The new form is described by M. F. Glaessner in the *Transactions of the Royal Society of South Australia* (81, 185; 1958) and has been named *Spriggina floundersi*. It represents a new family of the polychaete annelids. The body is flat, the head being without external segmentation and having lateral extensions giving it a roughly horseshoe shape, the trunk consisting of a very gently tapering series of segments with appendages terminating in bristles, numbering up to about 40 and not differentiated into a tail. The largest specimen measured 46 mm. long and 11 mm. wide. Since fossil jellyfish are found at the same locality it is not necessary to assume that *Spriggina* was hard-bodied. Certain features of the head resemble those of the living group Tomopteridae, members of which are pelagic. It is accordingly suggested that the aberrant pelagic adaptations of the Tomopteridae may conceal more primitive characters. In particular the lateral extensions of the head of *Tomopteris* and its composite nature may foreshadow the formation of primitive arthropod head shields such as those of the Middle Cambrian proarthropod (trilobitomorph) *Marrella*, and of trilobite larvæ or primitive trilobites. This possibility does not seem to have been considered by the zoologists.

The Fairy Fly

Prestwichia aquatica, the fairy fly, is a hymenopterous insect which parasitizes the eggs of aquatic insects, especially water-beetles, including *Dytiscus marginalis* L. It was first discovered by Sir John Lubbock in 1864 in a pond in Kent and was not seen again for thirty years, when Frederic Enock found a few specimens in a horse-pond. In 1896, and again in 1898, Enock records that he was able to find aquatic insect eggs containing both male and female specimens of *Prestwichia aquatica*, which eventually he reared. The eggs were taken from the previous year's stems of the water-plaintain, *Alisma plantago-aquatica* L. In a communication to the *Journal of the Quekett Microscopical Club* (28, No. 4; 1958), C. I. Ison writes that, since the work of Enock, *Prestwichia* has probably been found only on two occasions in Great Britain. The most recent was at Loughton, Essex, on July 17, 1958, when Ison saw two female fairy flies emerge from an unidentified egg. The males are much slower in emerging. Subsequent observations showed that twenty-six days elapsed before any movement of the parasites was noticed in the host egg after oviposition, and it was twenty-eight days before any females emerged. From one 2-mm. egg of the beetle, *Ilybius ater*, five male and twelve female fairy flies emerged. Observation of *Prestwichia aquatica* at the surface film of the water showed that they are quite at home whether they are above the film in the air or below, in the water, walking upside down.

Predatory Animals in Spain

During the past four years organized campaigns against predators have been taking place in Spain (*Oryx*, 5, No. 1; April 1959). Among the 100,000 mammals and birds killed were 36 martens, 1,051 wild cats, 55 lynxes, 380 badgers, 7,250 eagles and other birds of prey. Such indiscriminate slaughter can only lead to extermination of the rarer species, especially, for example, of the southern race of the Spanish lynx, which is chiefly a lowland animal. Both the imperial eagle and the short-toed eagle, which lays only one egg, nest in easily accessible places and are especially vulnerable. In Spain there is as yet little interest in the preservation of wild life, and the conservation laws are not enforced effectively. Every predator is believed to be an enemy. A new conservation law will remove both the lynx and the lammergeier from the list of vermin, and will give the lammergeier complete protection for five years.

Banana Wilt Investigations

In extended investigations of the vascular wilt disease of bananas caused by *Fusarium oxysporum* f. *cubense*, R. H. Stover has examined the evidence for clonal differentiation among wild type isolates of the pathogen (*Canad. J. Bot.*, 37, No. 2; 1959). *F. oxysporum* f. *cubense* exists in diseased rhizomes and pseudostems of Gros Michel bananas in two major clonal groups, or cultivars, designated 'Odoratum' and 'Inodoratum', because of the presence or absence of a distinctive odour from cultures after 4–8 days on various media. Three clones of the 'Inodoratum' cultivar, which are difficult to distinguish morphologically on peptone dextrose agar, were, however, distinguishable by cultural interaction and pathogenicity tests in the field. Also, some clones of the 'Inodoratum' and 'Odoratum' cultivars have been shown to differ quantitatively in pathogenicity in the field. Clone *C* from Honduras, of limited distribution, induced the most rapid disease-spread and destruction of banana mats, followed by clone *E* from Panama. There was an association between the 'Inodoratum' and 'Odoratum' cultivars and the syndromes induced in Honduras: the former usually induced strong yellowing of erect leaves, whereas the latter usually induced leaf collapse at the petiole with little or no leaf yellowing.

Exhibition of Old-time Beekeeping

An interesting exhibition of equipment used by old-time beekeepers in connexion with their ancient craft is open until the end of September at the

Museum of English Rural Life, Whiteknights Park, Reading. This, probably unique, collection of log hives, skeps of various materials and patterns, hackles, old style wooden hives of various kinds, honey and wax extractors, drone traps, feeders, smokers, queen excluders, etc., has been collected during the past few years by the Bee Research Association and is on permanent loan to the Museum. Entrance is free and a useful descriptive catalogue can be obtained from the Museum.

International Conference on Fishery Statistics

An international meeting of experts on fishery statistics in the North Atlantic area is to be held in Edinburgh during September 22–30. The conference is being convened by the Food and Agriculture Organization of the United Nations in co-operation with the International Commission for the North West Atlantic Fisheries and the International Council for the Exploration of the Sea. Governments concerned with the North Atlantic fisheries have been invited to nominate experts directly concerned with collecting, analysing and publishing statistics on the fishing industries and trades of their countries, and the Scottish Home Department will act as hosts to the delegates. It is hoped that delegates will be able to identify the main difficulties encountered in providing statistical data for the use of Governments, international agencies and the fishing industry, and will indicate the action to be taken to eliminate or reduce such difficulties.

The Marine Biological Association of India

The Marine Biological Association of India was founded at Mandapam Camp, to promote interest in marine biological and cognate sciences. The Association was formally inaugurated by the Hon. Mrs. Lourdhammal Simon, Minister for Fisheries and Local Administration, Madras State, on January 3. The following officers were elected: Dr. S. Jones (president), Prof. R. V. Seshaiya (vice-president), Dr. R. Raghu Prasad and Dr. C. P. Gnanamuthu (secretaries), Dr. R. P. Varma (assistant secretary), Shri K. V. Rao (treasurer), Dr. P. N. Ganapati (editor), Dr. R. Subrahmanyan (joint editor), and Dr. S. V. Job (managing editor). The official organ of the Association, the *Journal of the Marine Biological Association of India*, is expected to be issued half-yearly. Membership is open to all who are interested. All correspondence should be addressed to the Secretary, Marine Biological Association of India, Marine Fisheries P.O., Mandapam Camp, South India.

University News: North Staffordshire

The following appointments, among others, have been made: Mr. E. M. Hugh-Jones, fellow and tutor of Keble College, Oxford, professor of economics, in succession to Prof. B. R. Williams; Miss A. Eden, senior lecturer in education, Homerton College, Cambridge, lecturer in education and women's warden; Dr. V. C. Ewing, pressed steel research fellow, University of Oxford, lecturer in chemistry; M. F. Newman assistant lecturer in mathematics.

Announcements

An international conference on High Energy Accelerators and Instrumentation will be held at CERN during September 14–19. Further information can be obtained from the Conference Secretariat, CERN, Geneva 23, Switzerland.

The thirteenth congress of the International Scientific Film Association is to be held in London and Oxford during September 23–October 2, under the auspices of the British Scientific Film Association. Further particulars can be obtained from the Association's office at 3 Belgrave Square, London, S.W.1.

The International Council for Building Research, Studies and Documentation is organizing an international congress, to be held in Rotterdam during September 21–25. The topics for discussion include the design and calculation of structures; research problems relating to the application of heavy concrete elements; and fundamental aspects of the transmission of knowledge. Further information can be obtained from the C.I.B. Secretariat, c/o Bouwcentrum, P.O. Box 299, Rotterdam.

The Dublin and District Section of the Society of Chemical Industry, in conjunction with the Agriculture Group of the Society, is to hold a symposium in Dublin during September 15–18. The subject will be "Chemical Aspects of the Production and Use of Grass", and it is intended that the meeting shall include the presentation and discussion of pre-printed papers, and lectures and visits to places of scientific interest. Further information can be obtained from the honorary secretary, R. O. V. Lloyd, Chemist's Laboratory, A. Guinness Son and Co. (Dublin), Ltd., St. James's Gate, Dublin.

The Electrical Engineering Department of the University of Birmingham is arranging two informal residential conferences in September 1959. The first, on dielectric devices, will be held during September 14–17, and the second, on modern network theory, during September 21–24. Preliminary programmes of papers and discussions have already been arranged. Further information can be obtained from the Secretary of the Electrical Engineering Department, The University, Birmingham 15. In order to preserve the informal nature of the conferences, numbers will have to be strictly limited.

The fourth annual conference of the British Society for the Philosophy of Science will be held at Newnham College, Cambridge, during September 25–27, when the following papers will be read: "Scientific Research and the Philosophy of Science", by Prof. H. Dingle and Prof. H. C. Longuet-Higgins; "Classification, Concept-formation, and Language" by Miss M. Masterman and Dr. A. Koslow; "Knowing and Being", by Prof. M. Polanyi and Prof. R. B. Braithwaite; "Biology and Physics", by Prof. J. H. Woodger and Dr. E. H. Hutten. Further information can be obtained from the honorary secretary of the Society, Miss M. B. Hesse, University College, London, W.C.1.

The first Congrès International de Prophylaxie Criminelle will be held in Paris during September 27–30. Further information can be obtained from M. Stanciu, 7 Avenue Général Leclercq, Boulogne sur Seine.

Erratum. In the communication entitled "Possible Causes of Geomagnetic Fluctuations having a 6-Sec. Period", by H. J. Duffus, J. A. Shand and Sir Charles Wright, published in *Nature* of May 23, p. 1479, the caption for Fig. 3 should conclude: " . . . and of $\delta X/\delta t$ taken at Borrego, California, U.S.A.". The reference in the text to Fig. 3 should read: "Fig. 3 shows the simultaneous records of $\delta Y/\delta t$ at our base station and of $\delta X/\delta t$ near Borrego Springs . . . ".

CO-ORDINATION CHEMISTRY

THE Fourth International Conference on Co-ordination Chemistry was organized by the Chemical Society and held in London under the sponsorship of the International Union of Pure and Applied Chemistry during April 6–11. Since 141 contributions were received (from twenty-three different countries) in addition to seven invited lectures, it proved necessary to hold three concurrent sessions for the reading of papers. The following report is therefore necessarily based on direct attendance at only a sample of the papers presented and otherwise on the preprinted abstracts.

Prof. K. Ziegler (Mühlheim) in the first of the general lectures began by giving an account of the various steps involved in the catalytic polymerization of olefines by aluminium alkyls and concluded with some remarks on the influence of titanium salts on this reaction. The latter topic was taken up in a paper by G. Natta and G. Mazzanti (Milan), who presented evidence on the structures of some mixed complexes of titanium and aluminium which may be involved. The mechanism of the reaction between aluminium alkyls and titanium halides was further discussed in a paper from Israel.

Dr. H. M. N. H. Irving (Oxford) reviewed the available information on the stability of metal complexes in solution and showed how some simple theoretical ideas could be used to establish useful correlations. Among the many interesting contributions on this topic one may mention a study of thio-complexes from G. Schwarzenbach's laboratory in Zürich, a paper on the heats of formation of ethylenediamine complexes by M. Campolini and co-workers (Palermo), a study of polynuclear copper (II) complexes of carboxylic acids by D. L. Martin and F. J. C. Rossotti (Edinburgh) and work on silver (I)–oxine complexes by D. Dyrssen and collaborators (Stockholm).

Prof. H. Taube (Chicago) reviewed recent work on the mechanisms of complex ion reactions in solution and described some entirely novel experiments of his own. He provided conclusive proof that during the course of the oxidation–reduction reaction between cobalt (III) and chromium (II) ions under appropriate conditions, electrons are transferred through the whole length of the terephthalic acid molecule, and further showed that if the methyl ester of terephthalic acid was present, it was hydrolysed in the course of the reaction. This work opens the way for a full-scale attack on the vexed problem of electron transport through conjugated systems, and should have important consequences.

Studies on the mechanisms of the photochemical reactions of complex ions were reported by S. T. Spees and A. W. Adamson (University of Southern California), who discussed the possible role of doublet states in the photochemistry of chromic complexes; another paper on this subject was given by D. R. Stranks and G. J. Weston (Leeds). Contributions on the detailed kinetics of the hydrolysis of chromic complexes were presented by J. Bjerrum and co-workers (Copenhagen), who infer the existence of the $[Cr\ en_2(enH)\ (H_2O)]^{4+}$ ion as an intermediate in the hydrolysis of $[Cr(en)_3]^{3+}$, and by H. L. Schläfer (Frankfurt am Main), who studied the equation of $[Cr\ Ox_2\ en]^-$.

Dr. Greenberg presented the results of some recent studies from the U.S.S.R. on the *trans* effect in the substitution reactions of platinum (II) which suggest that the experimental situation is more complicated than has been previously realized. The first studies of the kinetics of the substitution reactions of *bis*cyclopentadienyl titanium dihalides were reported by A. Jensen and F. Basolo (Northwestern University, Illinois). The exchange reaction of quadridentate complexes, particularly of nickel (II), were discussed by W. W. Fee and B. O. West (Adelaide), and the kinetics of the dissociation of complex cyanides and of ethylene–diamine nickel complexes by G. Emschwiller (Paris) and by A. K. Shamsuddin Ahmed and R. G. Wilkins (Sheffield), respectively.

Some of the liveliest discussions followed papers of a rather theoretical nature dealing with the spectra and magnetic properties of complexes, for example, a paper on the relation between the geometrical structure and the magnetic properties of nickel (II) complexes by G. Maki, and another on the effect of the thiocyanate ion on the spectra of metal ions by C. E. Schäffer (Copenhagen). An interesting account of the magnetic susceptibility of rhenium compounds was given by B. Jezowska-Trzebiatowska (Wroclaw). The variation of the magnetic susceptibility of vanadium (III) complexes with temperature was discussed by B. Figgis, J. Lewis and F. E. Mabbs (University College, London). Two papers, one by S. Minc and W. Libus (Warsaw) and the other by F. A. Cotton and R. H. Holm (Massachusetts Institute of Technology), dealt with the physical properties and relative stabilities of the various types of cobalt (II) complex which exist in solution, and two others, one by C. R. C. Coussmaker and collaborators (Oxford) and the other by A. Turco and G. Giacometti (Padua), considered the properties of the versatile complexes of nickel (II) with substituted phosphines.

The X-ray determination of the structure of a complex formed by azobenzene and arsenic trichloride was reported by E. Ferroni and P. Orioli (Florence); the cuprous–diazoaminobenzene complex was shown by I. D. Brown and J. D. Dunitz (Royal Institution, London) to be dimeric in the crystal and to involve an eight-membered ring containing six nitrogen and two copper atoms. M. R. Truter and her co-workers (Leeds) discussed the structure of β-diketo complexes of trimethyl platinum (IV). Other structures described included those of basic zirconium chromates and β-Zn(OH)Cl. An important paper by I. Linqvist (Uppsala) surveyed the structures of some addition compounds of phosphorus oxychloride and selenium oxychloride.

A further group of contributions dealt with the elucidation of molecular and electronic structure by means of infra-red and Raman spectroscopy. K. Nakamoto, P. J. McCarthy and A. E. Martell (Clark University, Worcester, Mass.) discussed the infra-red spectra of the metal complexes of β-diketones, while D. B. Powell and N. Sheppard (Cambridge) dealt with the metal complexes of amines and ethylenediamine and showed that for the latter both *cis* and *gauche* configurations of the ligand are possible. Infra-red spectra of heteropolytungstates

were reported by a group of workers from Moscow. Raman spectra of addition compounds of sulphur trioxide were reported from Amsterdam and of stannic chloride from Karlsruhe.

Among the many papers principally concerned with the preparation of new compounds and the elucidation of their reactions one can pick out only a few. S. Herzog and co-workers (Jena) described the extension of their work on dipyridyl complexes of metals in abnormally low valencies to the preparation of the diamagnetic $[Ti(dipyridyl)_3]^-$ ion; L. Naldini (Milan) described a series of salts of the low-spin (hexa-aryl isocyanide)–manganese (II) complexes; V. Cagliotti and co-workers (Rome) discussed the preparation and reduction of the oxygen adducts of cobalt (II)–histidine complexes.

Carbonyls of iridium (III), *iso*cyanides of rhodium (II) and phosphines of osmium (I) were reported by L. Malatesta and S. Sandroni (Milan), by Miss L. M. Vallarino (Milan) and by L. Vaska (Mellon Institute, Pittsburgh), respectively. Complex fluorides of molybdenum and tungsten and rhenium were discussed by G. B. Hargreaves and R. D. Peacock (Birmingham); R. Hoppe and co-workers (Münster) reported on a variety of zirconium, tin and manganese complex fluorides. Heteropolyacids and related polyanionic compounds were treated extensively by groups from Roumania, the U.S.S.R. and Sweden. The properties of volatile nitrato–iron complexes and copper perchlorates were described by C. C. Addison and N. Logan (Nottingham) and by B. J. Hathaway (Hull), respectively.

The subject most completely covered both in general lectures and contributed papers was that of organo–metallic compounds involving transition metals. Dr. E. O. Fischer (Munich) surveyed experimental work on compounds involving 'sandwich' bonding in a comprehensive and lucid fashion. Prof. R. Nast (Heidelburg) described in detail his important researches on the complex acetylides of the metals; the least stable it appears are spontaneously explosive and the more stable only pyrophoric. Dr. H. W. Sternberg (Pittsburgh) discussed recent experimental work on the reactions of acetylenes, etc., with metal carbonyls and produced some interesting speculations on the relevance of these results to problems both of homogeneous and heterogeneous catalysis. Dr. L. E. Orgel (Cambridge) discussed bonding in zero-valent metal compounds.

Determinations of the crystal structure of some organo–transition metal complexes were reported by A. A. Hock, O. S. Mills and G. Robinson (Manchester); the results were, as usual in this field, very surprising. The infra-red spectrum of chromium dibenzene was described by A. Fritz (Munich). The symmetry of this molecule was discussed at some length. The view of the German school was that theory predicted it to have only three-fold symmetry as argued by E. Ruch (Munich) in a contributed paper. A number of other theoretical chemists (the present author included) held that no definite prediction could be made, but strongly favoured the view that the symmetry would prove to be six-fold. The experimental evidence, it seems, is not yet unambiguous.

A paper by L. S. Meriwether, Marilyn Fiene and G. W. Kennerly established that substitution reactions of a number of nickel carbonyl–phosphine complexes occur by way of a three co-ordinated intermediate rather than by a second-order mechanism. The reactions accompanying the polarographic reduction of iron carbonyls were considered by A. A. Vlček (Prague). There was some discussion of a paper by D. A. Brown (Queen Mary College, London) on the effect of phenyl-substitution on the stability of sandwich compounds.

The preparative work described in the field of acetylene–carbon monoxide chemistry was varied, fascinating, and still largely incomprehensible. G. Natta and co-workers (Milan) discussed the synthesis of succinic acid from carbon monoxide, acetylene and water in the presence of metal carbonyls. They suggested that it required binuclear metal complexes as intermediates and provided good evidence for the occurrence of a peculiar lactone-like derivative of succinic acid as a ligand in certain metal complexes. W. Hübel and E. H. Braye (Brussels) described the numerous new compounds which they have prepared from iron dodecacarbonyl and acetylenes. Some compounds contain aromatic rings, some contain cyclopentadienone, and others cannot at present be formulated in an acceptable fashion (like $C_2H_2Fe_2(CO)_5H_2$, they probably do not have 'acceptable' structures). E. Weiss and W. Hübel described the reactions of cyclopentadienones with metal carbonyls. They demonstrated the ability of cyclopentadienone in certain sandwich compounds to behave more like the anion derived from a phenol than like a normal ketone. Presumably this is due to charge-transfer from the metal to the oxygen of the ring.

The insertion of carbon monoxide into organic molecules by way of metal carbonyls was part of the theme of some of the general lectures. It was discussed in detail for the case of alkyl and acyl manganese carbonyls by T. H. Coffield and co-workers (Ethyl Corporation Laboratories, Detroit), who described some carbon monoxide exchange studies which throw considerable light on the reaction mechanisms involved. L. Kirch and M. Orchin (Cincinnati) described recent experimental work on the oxo-reaction.

Contributions on other metal alkyls included a discussion of the reactivity of the cadmium compounds by J. Vène (Rennes), a paper on alkyl and aryl palladium compounds by G. Calvin, G. E. Coates and P. S. Dixon (Durham), and another on the determination of Grignard reagents by F. G. Mann and I. T. Millar (Cambridge).

A group of papers described catalytic reactions other than those in the carbonyl field. One may mention that of W. Brackman (Royal Dutch Shell, Amsterdam), who described a series of copper–amine complexes which catalyse the oxidation of alcohols, and that of M. T. Beck (Szeged), who described much interesting work on catalysis that has been carried out in Hungary.

Of the papers not directly concerned with metal chemistry I shall mention one, the presidential address of the Chemical Society, which was open to all members of the Congress. Prof. H. J. Emeléus outlined the experimental situation in the field of inorganic polymeric molecules and amply illustrated his thesis that this is a fascinating and largely unexplored region of inorganic chemistry.

The Conference, seen as a whole, left one with no doubt that co-ordination chemistry has entered a period of rapid growth and is already invading fields which traditionally were distinct from it. The development of the various syntheses of large conjugated molecules from carbon monoxide and acetylene can scarcely fail to influence organic chemistry. The close contact between ligand field

theory and experiment in the field of co-ordination chemistry provides, perhaps for the first time, a climate in which experiments are designed to test the quantitative predictions of electronic theory; this must prove beneficial to both.

The organization of the Conference, both of the scientific meetings and the more social occasions, was uniformly excellent. All those who were present must be grateful to the members of the Chemical Society committees who were responsible for it and to those industrial concerns which, by their financial support or by acting as hosts, helped to make the meeting so successful.

L. E. Orgel

THE BRITISH COAL UTILIZATION RESEARCH ASSOCIATION

TWENTY-FIRST ANNIVERSARY OPEN DAYS

THE aim of the Association, from the time of its inception in 1938, has been "to promote research and other scientific work in connexion with the utilization of coal and its derivatives". Some of the achievements of twenty-one years were displayed at the research station during April 22–24.

In 1938, nine experimentalists worked in a single room, 12 ft. square, in Victoria Street, London. To-day the British Coal Utilization Research Association occupies a 20-acre site at Leatherhead, and is one of the largest of the thirty-nine industrial research associations supported by the Department of Scientific and Industrial Research; of its total staff of some 300, eighty are graduates or equivalent and a similar number are research assistants, representative of most of the recognized fields of physical science, from mathematics through the several branches of chemistry and physics to large-scale engineering.

The interests of the Association are many-sided, and this was reflected in the distinctive character of each of the three open days. April 22 was primarily 'members' day'; through its membership the Association forms a link between the four nationalized industries (coal, gas, electricity, transport) and about 150 other members (comprising individual firms and trade associations) which include industrial appliance makers, coal distributors and coal consumers. The Minister of Power, the Right Hon. Lord Mills, was the guest of honour, and in addressing a large and distinguished gathering at luncheon, he observed that the occasion marked the coming-of-age of an Association which had long since established itself as one of the most important and progressive industrial research organizations in Britain.

On the second day the guests were mainly drawn from the field of research; Government departments, research associations, universities and technical colleges were strongly represented. On the morning of the final day parties from local colleges and schools predominated, thus emphasizing the practical interest taken by the British Coal Utilization Research Association in educating young people in the science and technology of coal. In the afternoon the Association was honoured by a visit of a party from the London Section of the Royal Institute of Chemistry; and the day was fittingly concluded by a lecture to this party and other chemists by Prof. R. G. W. Norrish on recent work at Cambridge on flash photolysis in gases.

Throughout its history the British Coal Utilization Research Association has been in close touch with university thought and investigation and has itself maintained a strong programme of basic research. One well-known example of the effectiveness of this approach is the outcome of the experiments on coal breakage which were started in that small overcrowded laboratory in London in 1938. After some years of investigation, in collaboration with the British Colliery Owners Research Association, a specification for grading coal by size was drawn up, and this has been accepted by the National Coal Board as a basis for preparing and marketing coal. Another example of the application of basic research, also exhibited in the Physics Department, was the cone mill. Following a survey of the factors determining the power required for grinding coal to a given size, determination of the Hardgrove grindability index, and measurement of the intrinsic physical and mechanical properties of coal, a matrix theory of coal breakage was developed and the first cone mill was built to test this theory: now its engineering utility is being studied.

Some of the modern techniques being used to investigate both the chemical and the physical aspects of coal constitution were displayed. Research into the chemical basis of the plastic softening of coals, with the view of achieving better control of coking properties, has recently made considerable progress, and current knowledge of the mechanism was illustrated diagrammatically alongside the special apparatus employed. Another exhibit was of interest as the first complete conversion, in a single step, of coal to chemical products without simultaneous production of tar or solid residue: low-rank coals have been treated with chlorine trifluoride, producing a range of chlorofluorocarbons (from colourless oils to resin-like solids) possessing chemical and physical properties that indicate their possible suitability for certain industrial applications.

Considerable work by the Association over a number of years on the ultra-fine porous structure of coal and its development during carbonization has led to the placing, by the U.K. Atomic Energy Authority, of a research contract for fundamental work on the graphite used as moderator in nuclear reactors. This investigation has already shown that closed pores in the graphite become accessible during its reaction with oxidizing gases, and an estimate of the importance of internal burning has been obtained.

Among the current major problems facing the coal industry are: improvement of methods for using small coal; meeting the challenge of competition from oil; and assistance in the implementation of the Clean Air Act. Research by the Association has, therefore, been directed especially towards: (*a*) improving and devising appliances suitable for burning small coal; (*b*) developing methods for the automatic control of combustion; and (*c*) investigations on reduction of atmospheric pollution from coal-burning appliances.

One of the most important current needs is a method of converting small coal cheaply to gas, and ultimately

to oil. The Association's experimental slagging gasifier, built under contract with the Ministry of Power, at present consumes about a ton of fuel an hour and can take, in short tests, up to half of this in the form of pulverized coal. It has been operated successfully at various ash-levels and over a range of ash fluidities. Background studies have made possible the tentative correlation of slag viscosity–temperature curves with slag composition; and improved methods of analysis have enabled the important constituents of ash to be determined at the rate of four samples a day.

The automatically controlled small-pipe, forced-circulation central heating system, developed by the Association, is being actively promoted by the Coal Utilization Council, and was displayed by them in the exhibition they had arranged on the site. Also on view in the Council's exhibition were some of the thirty or more designs of free-standing convector fire with restricted throat, a type of appliance that has been developed by the British Coal Utilization Research Association.

The Association's Calorimeter Room has taken its place as an absolute standard for testing domestic appliances. A method of automatic control for miniature chain-grate stokers, used to fire central-heating boilers (½–2 million B.T.U./hr.) in offices or blocks of flats, has been devised, and a unit burning ½-in. small coal smokelessly and at high efficiency is now being tested.

An air–fuel ratio controller has been developed within the Association for fitting to stokers of Economic and Lancashire boilers in order to take much of the responsibility for maintaining the correct ratio of fuel to air out of the hands of the fireman, and one of these controllers was in use on the boiler used for site heating.

A first step towards finding means for reducing atmospheric pollution from coal-burning appliances has been made through the work on the measurement of dust and grit emission from boiler furnaces. The methods of sampling dust from stacks developed by the Association were on view and have aroused much interest in those concerned with enforcing or complying with the Clean Air Act. The automatic particle-sizing apparatus developed by the Association was displayed; with special care it can be employed down to 2 microns and can be used to carry out eight analyses a day.

The study of problems arising from the deposition of inorganic solids and corrosive liquids from flue gases in water-tube boilers forms an important part of the Association's work; these problems are investigated both in the laboratory and at power stations. Large- and small-scale equipment used in these investigations was on view. Special measuring apparatus devised for this work was also demonstrated.

It has not been possible in this short account to mention more than a selection of the exhibits. A fuller account of the current researches is contained in the annual report of the British Coal Utilization Research Association for 1958, and a bibliography of the Association's publications during 1938–59 is to be published in the near future.

THE EAR UNDER WATER

A DISCUSSION meeting devoted to "The Ear under Water" was held at the Royal Society on March 12. In his opening survey, Prof. O. Lowenstein pointed to the fact that even in its most highly evolved state the vertebrate ear is a dual—or even triple—purpose sense organ. It incorporates receptors for angular acceleration (semicircular canals), for linear acceleration including gravitational stimuli (otolith organs) and for oscillatory changes in linear acceleration such as vibration in general and sound in particular (otolith organs, cochlea). The vertebrate ear appears to be a phylogenetic novelty derived by modification from the lateral-line organs of early fish-like vertebrates and not from the statocyst of the invertebrates. Thus, the ear is probably not the comparative-anatomical homologue of the invertebrate statocyst. There is no doubt, however, that the two organs are functionally analogous. It was therefore considered desirable to review recent work on the invertebrate statocyst in the context of the symposium.

Dr. Melvin Cohen (University of Oregon) described the anatomy of the statocyst of the lobster, *Homarus americanus*, and discussed the results of an electrophysiological analysis of its function. It contains two types of position receptors, one for the monitoring of absolute position and one which also indicates the direction from which a certain position is approached. A third receptor type functions as an acceleration receptor and is insensitive to position. There are receptors responding to vibration of the solid substrate, but no reactions to air- or water-borne sound could be registered. Dr. Cohen pointed to the striking similarities between the responses from the crustacean statocyst and those from the various end organs of the vertebrate labyrinth.

In a paper on the cephalopod statocyst, Prof. J. Z. Young gave a detailed description of the morphology of the statocyst of the octopus. This remarkable sense organ, too, shows a most interesting and functionally suggestive number of items of structural convergence with the vertebrate labyrinth. Apart from a vertically placed macula with otolith, there is a crista which has sections in three planes of space, each section being associated with a separate nerve. The sensory hairs are ensheathed in a matrix of unknown composition forming cupulæ reminiscent of those found in the labyrinth. Large neurons having dentritic endings as well as hair processes underlie the sensory cells of the crista and appear to make synaptic contact with them, although the hair cells themselves are primary sensory cells equipped with centripetal axons. A hand-shaped vane (the so-called anti-crista) protrudes into the endolymphatic space and seems to be so arranged as to protect the vertical section of the crista from overstimulation when the octopus suddenly accelerates forward, as it does on attacking prey.

Prof. Young stressed that there is no behavioural evidence of hearing in the cephalopods, but on anatomical grounds there is no reason why the cephalopod statocyst should not be potentially capable of vibration reception or even hearing, besides its manifestly obvious function as an organ of static and dynamic spatial orientation.

The vertebrate ear evolved in aquatic animals, and it may well be that its function as a vibration receptor evolved simultaneously with that of an organ of orientation. As an introduction to the problems connected with hearing in aquatic vertebrates, Dr. P. Vigoureux (National Physical Laboratory, Teddington) gave a most useful survey of the physical aspects of sound propagation under water. So far as penetration of sound from air into water is concerned, there is a critical angle of 13° of reflexion at the interface between air and water, and total reflexion occurs at all greater angles of incidence. Sound can therefore penetrate into water only if its incidence is almost vertical. Yet the human ear is sensitive enough to hear under-water sound from a powerful source in the air overhead. Dr. Vigoureux also discussed some effects of temperature gradients leading to conditions of exceptionally good sound propagation over long distances. Sound absorption is only 1/1000th part of what it is in air, and this is yet another reason for the considerably greater distance of sound propagation in water.

Prof. S. Dijkgraaf (Utrecht) introduced his account of hearing in bony fishes by a definition of sound and hearing. This has for a long time been a controversial matter and he advocated ascription of the ability of hearing to such animals as are sensitive to a succession of pressure waves propagated with a characteristic velocity through the medium, be it air or water, and able to detect these stimuli by means of special receptors exclusively used for this purpose. If the latter condition is not fulfilled, one should speak of sound- or vibration-reception instead of hearing.

Prof. Dijkgraaf distinguished among the bony fishes between what he called 'specialists' and the normal run of fishes. The 'specialists', mostly freshwater fishes, have been shown to possess sound-reinforcing structures like the Weberian ossicles or special gas chambers connected with the ear. Their frequency-range is considerable and they are more highly sensitive to tones than man up to a frequency of 1,000 c./s. They are also capable of pitch discrimination over a limited range (up to 1,260 c./s.) and can discriminate frequency differences as low as 4 per cent. However, no evidence for directional hearing has so far been obtained. So-called normal fishes (almost all marine forms) have an upper limit of 400–1,000 c./s. and a much higher threshold.

In the second part of his paper, Prof. Dijkgraaf dealt with the problem of functional localization of hearing within the labyrinth of bony fishes and discussed the part played by the otolith organs in the sacculus and lagena in some, and the probable role of the utriculus in other forms, depending on differences in the nature and topographic deployment of the sound-reinforcing structures. Tactile skin receptors may be involved in the reception of low-frequency sound, but there is no evidence for the collaboration of the lateral line.

In higher vertebrates the ear became adapted to the reception of airborne sound. The special matching devices of the middle ear and the range-finding pinna of the mammals were evolved. Papers by Dr. Reysenbach de Haan (Eindhoven) on aspects of mammalian hearing under water and by Dr. F. C. Fraser and Mr. P. E. Purves of the British Museum on the osteology, acoustic matching, and evolution of air sinuses, and on muscular and vascular arrangements, acoustic isolation, and directionality of hearing, dealt with the way in which the Cetacea solved the problems of underwater hearing on their return to a fully aquatic existence.

Dr. Reysenbach de Haan pointed out that under water the external ear and the middle ear of the terrestrial mammal become useless. Man has no directional hearing under water and suffers a loss in hearing of 60 db. Whales and dolphins rely chiefly on sound for their orientation, and yet they have no outer ears and their auditory meatus has become highly modified. Dr. Reysenbach de Haan believes that the modified structures corresponding to the meatus are devoid of function, as the rate of transmission of sound in the tissues around the external auditory canal is practically equal to that in water. The bulla, however, is filled with air and kept hermetically sealed from the water outside. The striking feature of the middle ear or bulla is that it has parted company with the skull and, like the inner ear in the petromastoid, has come to hang loose under the skull. Foam-filled sinuses in the petromastoid are in communication with the air of the middle ear in the bulla and via the Eustachian tube with the air of the respiratory passages. The inner ear is thus completely air-insulated from the surrounding tissues, from the skull, and from the surrounding water. The two ears are also completely insulated from one another. This, Dr. Reysenbach de Haan held, was an indispensable condition for successful directional hearing under water.

Modifications of the middle ear and also of the cochlear structures were demonstrated to be associated with the sensitivity of the Cetacea to supersonic frequencies higher than 100,000 c./s. Cetaceans produce high-pitched sound and are known to echolocate like bats. In water, with the four times longer wave-length, much higher frequencies are necessary for the detection of objects of the same size.

Experiments on the directional hearing of dolphins and the methods used in the capture of these animals in Danish waters and their transport to the Netherlands were illustrated by a film made by W. H. Dudok Van Heel (Holland).

In their communication on the anatomy and function of the cetacean ear, Dr. F. C. Fraser and Mr. P. E. Purves, of the British Museum of Natural History, dealt with the morphology of the auditory meatus in toothed whales and whalebone whales. They have come to the conclusion, contrary to the opinion voiced by Dr. Reysenbach de Haan, that the meatus is not devoid of function. They believe that it plays an important part in sound conduction.

Experiments on a large portion of the squamomastoid region of a fin whale, preserved by deep-freezing and dissected after thawing to expose the middle ear, the wax plug and meatus, showed that the attenuation of high-frequency sound waves (100,000 c./s.) was considerably lower when conduction followed the meatus with its lengthwise orientated fibre system, than in the surrounding tissues in which the fibrous structures are randomly orientated. The authors assumed that sound transmitted by the meatal path would be dominant at any level of intensity. The animal must be subjected to an intensity and/or phase difference at the cochleæ of the two ears due to the screening effect of the head and the distance separating the two meatal openings. This may be of vital importance for directional hearing.

A detailed description of the air sinuses and peculiarities of the vascular system served to em-

phasize the acoustic isolation of ear from skull and jaws and ear from ear. The part played by the albuminous foam formed by nitrogen in an oil–mucus emulsion was discussed, and it was shown how this foam can remain persistent even during a prolonged dive.

Finally the modifications of the middle ear ossicles were described with reference to the requirement for successful matching of the cetacean ear to water-borne sound. They were illustrated by a working model.

A series of anatomical exhibits was shown to illustrate the specialization in the topographic relationship between ear and skull, especially the arrangement of air sinuses in the various cetaceans.

The contributions to the symposium will be published in full in the *Proceedings of the Royal Society*.

O. LOWENSTEIN

THE INDIAN ASSOCIATION FOR THE CULTIVATION OF SCIENCE

THE annual report for 1957–58 of the Indian Association for the Cultivation of Science*, which includes lists of papers published during the year under their departmental headings, records further work on the thermal diffusion and inter-diffusion of gases. An apparatus has been designed for measuring the thermal conductivity of diffusing gases and a new apparatus constructed for purifying high-pressure commercial hydrogen. The dimensions of the unit cell of naphthazarin have been re-determined by X-ray methods and further work on the molecular structure of this crystal is in progress. The work of the Department of Magnetism was reorganized and the study of paramagnetic anisotropy of dihalides of copper(II), the paramagnetic behaviour of fluosilicates and chlorostannates of copper(II) and measurement of the principal susceptibilities of paramagnetic crystals down to liquid-hydrogen temperatures has been undertaken, as well as the study of paramagnetic crystals both by the paramagnetic electron resonance and the proton resonance method. The anisotropy of many nickel(II) salts of similar constitution was studied to ascertain the effect of long-range crystalline fields in these salts, and work on paramagnetic alums of titanium(III), vanadium(III) and chromium(III) was completed.

In the Optics Department the Raman spectra of *o*-chlorophenol in different solvents, of α- and γ-picoline and their solutions in alcohol in liquid and solid state, and of ethylbenzene, chlorobenzene and bromobenzene in the frozen state at − 180° C. have been determined and the ultra-violet absorption spectra of many aromatic compounds studied in the vapour, liquid and solid states, as well as those of frozen 0·2 per cent and 25 per cent solutions of the dichlorobenzenes in *iso*-butyl alcohol at − 180° C. and the Debye–Scherrer patterns due to frozen toluene, chlorobenzene and bromobenzene and to diphenyl at 32° C. and at − 180° C. The Department of Theoretical Physics was concerned mainly with the spin of an electron from five-dimensional wave equations, the influence of constant electric and magnetic fields on the spin of the particle, electronic energy bonds in lattices and some generalizations in relativistic cosmology.

In the Department of Physical Chemistry, investigations in high polymer chemistry related to the kinetics and chain transfer behaviour of methyl *iso*propenyl ketone, the aqueous polymerization of vinyl monomers in redox systems, the thermodynamic properties of binary mixtures, the determination of absolute values of the rate constants in polymerization and the preparation and properties of soil conditions of the krillium type. In general and analytical chemistry, work is being carried out on the mutual solubilization of oils and water, the decomposition of peroxides in different solvents and the velocity of ultrasonic waves in solutions and liquids. An apparatus has been assembled for determining the mutual solubilities of organic compounds *in situ*. The Department of Organic Chemistry continued its investigations on sesquiterpenoids, diterpenoids, triterpenoids and steroids, and in the Department of Inorganic Chemistry attention was concentrated on the study of co-ordination complexes and their applications in analytical chemistry. It has been shown that 3- and 5-oximinomethylsalicylic acids and 3- and 5-aldehydosalicylic acids can be used for estimating thorium and its separation from the cerite earths.

* Indian Association for the Cultivation of Science. Annual Report for 1957–58. Pp. 52. (Jadavpur, Calcutta: Indian Association for the Cultivation of Science, 1959.)

THE EAST AFRICAN INDUSTRIAL RESEARCH ORGANIZATION

REPORT FOR 1957–58

THE annual report for 1957–58 of the East African Industrial Research Organization* records the official opening of the new headquarters of the Organization in Nairobi on February 14, 1958, and spectacular results from a close collaborative study by the Fuel Advisory Service with the Research and Economy Section of the Chief Mechanical Engineer's Department of the East African Railways and Harbours, notably in the design of a special burner for lighting up locomotive boilers and in increasing the power developed by locomotive engines in service, using a Giesil ejector. Advice was also given on the utilization of wood waste, and fuel economies of 5–20 per cent were demonstrated in some thirty factories. An oil-fired incinerator was designed for the new swine fever research centre at Mugaga and a similar but larger one for the new Wellcome Foot-and-Mouth Disease Centre.

The Department of Fuel Technology and Chemical Engineering has also examined the use of solar driers and the dust-proofing of motor cars. Extensive work on the mechanical drying of Arabicz coffee has shown that damage is reduced by pre-drying and increased by increasing the final drying temperatures, and that daylight has a marked effect on the liquoring quality

* East Africa High Commission. East African Industrial Research Organization Annual Report, 1957–1958. Pp. ii+18. (Nairobi: Government Printer, 1958.) Sh. 2.

of the dried bean. The range of light frequencies responsible is now being studied, and apparatus has been constructed for the determination of moisture content and desorption isotherms using a wet- and dry-bulb technique with recirculated air flow. Two full-scale driers for use on wet groundnuts were designed and tested for the National Institute of Agricultural Engineering and the Tanganyika Agricultural Corporation.

Work on the production of vitreous bowls should now permit costs of production to be estimated. Successful large-scale trials of locally made refractory bricks and bonding cement for setting them in locomotive fire-boxes are reported. Much work was done on the elimination of arsenic from cement copper prepared from an ore containing arsenic and on the production of high-grade silicophosphatic fertilizer by the low-soda process.

PART-TIME WORK FOR WOMEN GRADUATES

IN 1956, as an experiment, an agency was started in the London area to put women graduates in touch with any suitable part-time work. From the beginning it was hoped that the agency might not only help to canalize existing opportunities but also increase them. Many potential employers are prejudiced against part-time workers; it was felt that they might have more confidence if they could interview not one but a number of suitable candidates for each vacancy, and feel that they could call on a reserve if the need arose.

Some types of work, such as highly responsible administrative posts, could not be adapted to part-time hours, but other types could: teaching, research assistance, information work, indexing, cataloguing, translation, abstracting, statistical work all seemed possible to arrange in this way. It also seemed clear that much of this work could be done at home and that it would be greatly welcomed, especially by mothers of young children. The growth of the agency has recently been described by Mrs. D. D. Furley (*Univ. Rev.*, 31, No. 2; February 1959).

The agency started in a modest way with a nucleus of about twenty graduates in London who were in search of part-time work. At first, few vacancies were notified to the agency. After the appearance of one or two letters to the Press from people who had heard of the scheme, about three hundred graduates registered as potential part-time workers in the London area and letters were received from about five hundred others living outside the London area who were seeking work, but did not realize that the scheme applied only to London.

Many employers were sympathetic to the idea and more jobs gradually became available.

In the first eighteen months, 378 jobs were notified to the agency, and 260 of them were filled by graduates registered with it. In the following twelve months, 440 jobs were notified, and 303 were filled. The majority of these jobs were in teaching, research of one kind or another and editorial or secretarial work. The figures included both temporary and permanent jobs; several women who started by filling some temporary vacancy have later been found permanent part-time jobs by the same employer.

It is impossible to fill some of the vacancies notified to the agency. There are many reasons: part-time science teachers are as rare as full-time ones; there is more demand than supply for good secretaries; the pay offered is sometimes, and particularly in social work, unattractive; and vacancies are often separated from the likeliest candidates by a journey of impossible complexity and length.

Fewer permanent than temporary jobs are filled. This seems to reflect a general reluctance on the part of married women to commit themselves to permanent work. Women who have not held a job since marriage or since having children are sometimes uncertain how it will work out. Mothers are always reluctant to commit themselves to working during school holidays and permanent jobs which conform to school holidays are very hard to find.

In May 1957 a similar agency was opened in Manchester. Although similar to the London agency, in one way it differs from it. As Manchester is a smaller and non-metropolitan city, it was thought advisable not to confine the agency to graduates, but to use it for other women with comparable qualifications—diplomas, teachers' training certificates, or knowledge of foreign languages. Eighteen months experience in Manchester bear out the findings of the London agency.

Altogether, more than a thousand women, about 800 of them married, have registered with the London agency since its inception. Although about 40 per cent of them have been 'housebound' by children, illness or elderly relatives, approximately half of them have found work through the agency. In Manchester more than one hundred women have registered, all but nine of whom are married; sixty of these have a university degree, twenty-three a diploma of one kind or another. Forty-six of these have found temporary or permanent work through the agency.

MIGRATION AND DISPERSAL OF NEW ZEALAND GANNETS

A TOTAL of 3,999 gannet chicks was banded at Plateau station, Cape Kidnappers, Hawke's Bay, and Horuhoru station, Hauraki Gulf, New Zealand, between 1951 and 1957. Up to March 31, 1957, 207 recoveries were made. These recoveries include 26 chicks which had not left the gannetry, 146 birds recovered outside the gannetry, and 35 live subadult birds caught on their return to Plateau gannetry. An account of the investigation has been given by Kazimierz Wodzicki and Peter Stein (*Emu*, 58, Part 4; September 1958).

The percentage of recovery for the whole period was 3·1 (3·3 from Plateau and 2·9 from Horuhoru). This distribution of recoveries throughout the year shows two periods of increased mortality; one affecting first-year birds within the first few months

of their independent life and associated with the crossing of the Tasman Sea; another peak occurring in late spring and summer and comprising one- to two-year-old birds.

It was found that fully fledged chicks leave the gannetry at the age of 15½ weeks. The majority of Plateau gannets take a northern route towards the North Cape; others migrate along the eastern coast of North Island and reach Australia through Cook Strait. Recoveries of Horuhoru gannets show an almost exclusive use of the northern route. At the age of 24 weeks all but sickly or late chicks cross the Tasman Sea with an average speed of up to 240 miles a day.

In Australia young gannets disperse widely from subtropical waters in the south and to the Indian Ocean pelagic waters in the west. Horuhoru birds tend to go further south than those banded at Plateau. There seems to be no appreciable difference in geographical distribution among various age-groups of the sample. Sub-adult gannets remain in Australian waters until they are two to three years old.

From that time an increasing number of recoveries is made in New Zealand, where the birds disperse around the coast, and there appears to be some difference in the geographical distribution of recoveries from the two stations.

An analysis of live birds recovered at the gannetry shows that young gannets begin to reappear at their home-gannetry at the age of three years, first as 'roosting' or 'unemployed' birds; from the age of 4–5 years they commence to breed, but at the age of 6–7 years only about half of the birds are breeding.

In all three subspecies of the gannet there is a similar strong urge in juvenile birds to migrate to warmer seas; a similar maximum distance of about 4,000 miles may be flown by the three subspecies, but whereas North Atlantic and Cape gannets move towards the equator, New Zealand-bred Australasian gannets migrate westwards.

BIOSYNTHESIS OF CELL CONSTITUENTS FROM C_2-COMPOUNDS

Formation of Malate from Glycollate by *Pseudomonas ovalis* Chester

DURING the growth of micro-organisms, intermediates of the tricarboxylic acid cycle are utilized to provide the carbon skeletons of many cell constituents[1,2]. When C_2-compounds are the sole source of carbon, means must exist whereby such intermediates, drained from the cycle, are replenished by ancillary reactions. When the C_2-substrate for growth is acetate, this is accomplished via the glyoxylate cycle[3–5], in which the decarboxylative steps of the tricarboxylic acid cycle are replaced by the overall reaction:

$$\text{acetate} + \textit{iso}\text{citrate} \rightarrow \text{succinate} + \text{malate} \quad (1)$$

Reaction (1) is the sum of two reactions, catalysed respectively by *iso*citratase[6,7] and by malate synthetase[8]. Experiments on the influence of growth substrates on the formation of *iso*citratase[9] have shown, however, that this enzyme is formed only when acetate or a precursor of acetate (such as octanoate[10]) serves as growth substrate, and that cells grown on C_2-compounds other than acetate (such as glycollate, glycine or oxalate) contain only negligibly low amounts of *iso*citratase. Other routes must therefore exist for the necessary syntheses of tricarboxylic acid cycle intermediates from these C_2-compounds.

The purpose of this communication is to show that cells grown on glycollate contain enzymes capable of forming malate from glycollate, and to suggest a possible route (Fig. 1) for the formation of cell constituents from glycollate. The evidence for this suggestion rests on the following observations:

(1) Washed suspensions of *Pseudomonas ovalis* Chester grown on glycollate readily oxidized glycollate, glyoxylate, pyruvate and all intermediates of the tricarboxylic acid cycle, indicating the occurrence of that cycle under these conditions.

(2) Extracts of such cells, obtained by supersonic disintegration and centrifugation, readily catalysed the formation of glyoxylate from glycollate. Fractionation of such extracts with ammonium sulphate indicated that this enzyme, which catalysed the reaction:

$$\text{glycollate} + A \rightarrow \text{glyoxylate} + A.2\text{H} \quad (2)$$

where A is an unknown electron acceptor, was largely concentrated in that fraction precipitable between 0 and 35 per cent saturation with ammonium sulphate. The bright yellow colour of this fraction, and the finding that its ability to oxidize glycollate was enhanced by the addition of phenazine methosulphate (0·1 per cent w/v), suggest that reaction (2) may be catalysed by a glycollic oxidase, possibly similar to that described in spinach leaves[11], in which case the electron acceptor A would be a flavoprotein.

(3) When glyoxylate was incubated anaerobically with crude sonic extracts of cells grown in glycollate, carbon dioxide was rapidly evolved. In order to establish the stoichiometry of this reaction, the crude extracts were fractionated with ammonium sulphate (35–50 per cent saturation), dialysed, and eluted from columns of diethylaminoethyl cellulose with portions of phosphate buffer, *p*H 7·5, of increasing strength. When the fraction eluted by 0·1 *M* buffer was incubated with 6·7 μmoles of glyoxylate, 3·4 μmoles of carbon dioxide were evolved; this is quantitatively in agreement with the following reaction:

$$2\ \text{glyoxylate} \rightarrow CO_2 + C_3\text{-compound} \quad (3)$$

observed by Krakow and Barkulis[12] with extracts of *E. coli* grown in glycollate. As described by these authors, maximal enzymic activity depended on the presence of magnesium ions and thiamine pyrophosphate.

(4) This purified cell fraction also catalysed the oxidation of reduced diphosphopyridine nucleotide in a reaction dependent on the presence of glyoxylate and enhanced by the addition of magnesium ions and thiamine pyrophosphate. The rate of oxidation of reduced diphosphopyridine nucleotide increased with time, and was more rapid after pre-incubation of the cell fraction with glyoxylate, magnesium ions and thiamine pyrophosphate than when it was present from the start. This suggested that the

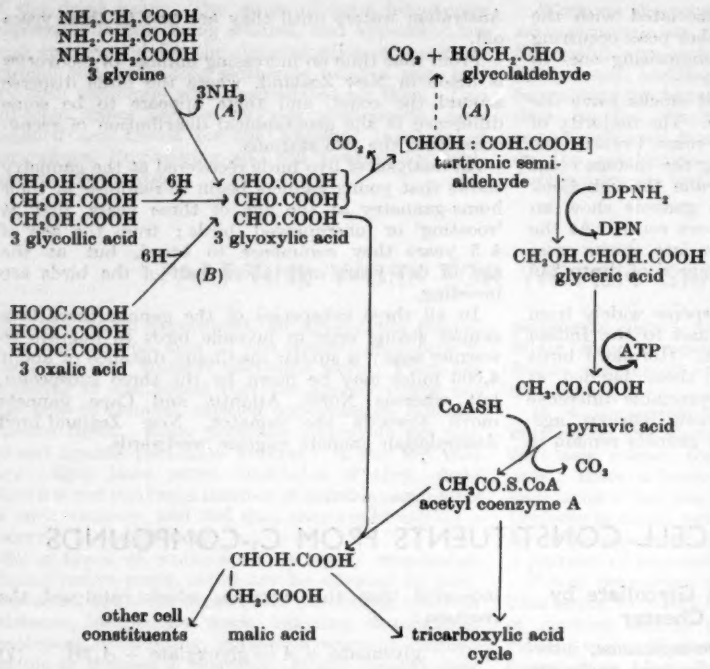

Fig. 1. Possible route for the formation of cell constituents from C_3-compounds by *Pseudomonas*
(DPNH₂ = reduced diphosphopyridine nucleotide; ATP = adenosine triphosphate; CoASH = coenzyme A)
(*A*) see Callely and Dagley (second communication); (*B*) see Quayle and Keech (third communication)

compound reduced by reduced diphosphopyridine nucleotide was not glyoxylate, but a material derived from glyoxylate.

(5) In the presence of thiamine pyrophosphate and excess reduced diphosphopyridine nucleotide, the purified cell fraction catalysed the formation of 1·8 μmoles of glyceric acid for the utilization of 3·5 μmoles of glyoxylate. No glyceric acid was formed when reduced diphosphopyridine nucleotide or glyoxylate was omitted, or when the cell fraction was boiled prior to incubation.

(6) In the absence of reduced diphosphopyridine nucleotide, the purified cell fraction catalysed the conversion of [1-^{14}C] glyoxylate to carbon-14 dioxide and an unknown material, which was capable of reacting with acid 2,4-dinitrophenylhydrazine to form a radioactive derivative. On addition of alkali, this derivative lost most of its radioactivity and gave a spectrum indistinguishable from that of the *bis*-2 : 4-dinitrophenylhydrazone of glyoxal (max. light absorption at 570 mμ). In the presence of reduced diphosphopyridine nucleotide, this unknown material did not accumulate; instead, 1 molecule of radioactive glycerate was formed for each molecule of carbon-14 dioxide evolved and for each 2 molecules of [1-^{14}C] glyoxylate utilized. No labelled glycollate was found, which showed that glyoxylic reductases[13,14] were absent from this cell fraction.

(7) The incorporation of label from [1-^{14}C] glyoxylate into glycerate by the purified cell fraction was not affected by addition of hydroxypyruvate, and the hydroxypyruvate re-isolated after the incubation was devoid of radioactivity. The purified cell extract did not catalyse the oxidation of reduced diphosphopyridine nucleotide in the presence of hydroxypyruvate.

These results indicate that the overall process occurring was the formation of a C_3-compound from glyoxylate by reaction (3), followed by its reduction to glycerate:

$$C_3\text{-compound} + DPNH_2 \rightarrow \text{glycerate} + DPN \qquad (4)$$

They further show that, unlike the system described by Krakow and Barkulis[12], the C_3-compound formed by *Pseudomonas* extracts was neither hydroxypyruvate nor any material in ready equilibrium with it. It is conceivable that the unknown C_3-compound is tartronic semialdehyde[15], the ene-diol form of which[16] (HCOH : COH.COOH) might exhibit the properties noted under sections (4)–(7) above.

(8) Crude sonic extracts of cells grown in glycollate formed carbon-14 dioxide and radioactive glycerate from [1-^{14}C] glycollate, this being the sum of reactions (2)–(4).

(9) Crude extracts of cells grown in glycollate prepared in a Hughes press[17] catalysed the formation of pyruvate from glycerate when incubated with adenosine triphosphate, but not when adenosine triphosphate or glycerate was omitted or when the cell extract was boiled prior to incubation.

(10) Crude sonic extracts of cells grown in glycollate readily oxidized pyruvate, presumably via acetyl coenzyme A. Such extracts also contained high activities of malate synthetase[8] and were capable of forming[18] about 7 μmoles of malate/hr./mgm. of protein from acetyl coenzyme A and glyoxylate, as measured by optical assay[19].

Reactions (2)–(4), in conjunction with the reactions leading from glycerate via pyruvate to acetyl coenzyme A, would represent the conversion of two molecules of glycollate to one of acetyl coenzyme A and two of carbon dioxide. Condensation of the acetyl coenzyme A thus formed with one molecule of glyoxylate (derived from the oxidation of a third molecule of glycollate) would yield one molecule of malate:

$$3 \text{ glycollate} \rightarrow \text{malate} + 2\ CO_2 + 6\ H \qquad (5)$$

which would serve as the required precursor of cell constituents. The findings described by Callely and Dagley and by Quayle and Keech in the communications following indicate that this or closely similar biosynthetic routes may operate also in pseudomonads grown on glycine or oxalate as sole carbon sources.

Full experimental details of this work will be published elsewhere.

We wish to thank Sir Hans Krebs for his interest and encouragement, and Prof. F. Dickens for gifts of Li hydroxypyruvate. This work was supported in part by the Rockefeller Foundation and by the

Office of Scientific Research of the Air Research and Development Command, U.S. Air Force, through its European Office, under Contract No. AF 61(052)-180.

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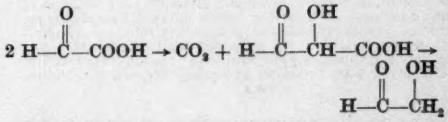
Metabolism of Glycine by a Pseudomonad

BACHRACH[1] isolated from the deep litter of a poultry house four species of *Pseudomonas* that utilized uric acid as sole source of carbon and nitrogen for growth. Prof. B. C. J. G. Knight kindly made these strains available, and we have studied the metabolism of glyoxylic acid by '*Pseudomonas A*' grown with glycine as sole source of carbon in aerated mineral salts media at 30° C. Whole cells oxidized various acids of the tricarboxylic acid cycle; and although citrate was attacked only slowly, this may be attributed to slow penetration of the cells by the compound, which was oxidized by cell-free extracts. Glyoxylic acid was oxidized rapidly by cells grown with glycine, but not with succinate as source of carbon. The work of Janke and Tayenthal[2] and Campbell[3] has established glyoxylate as an intermediate in glycine metabolism by species of *Pseudomonas*.

Extracts of cells grown with glycine catalysed the decarboxylation of glyoxylic acid. After disintegration in a Hughes press[4] cooled to −14° C., each 10 gm. of crushed cells was homogenized with 1 gm. of powdered alumina and 25 ml. of phosphate buffer, pH 7·2; cell debris and alumina were then removed by centrifuging to give a clear yellowish extract. Manometer flasks filled with nitrogen and fitted with two side-arms contained different amounts of glyoxylic acid (3–17 μmoles) to which 0·5 ml. of extract was added at 30° C. The carbon dioxide released after tipping 0·5 ml. of 3 *N* sulphuric acid from the second side-arm was about 85 per cent of that calculated on the assumption that one mole of glyoxylate gave rise to one mole of gas. Extracts did not oxidize glyoxylate: the same amounts of carbon dioxide were evolved in air as in nitrogen; and no glyoxylate or other keto-acid remained when all gas had been released. The reaction was also conveniently studied by following the rate of disappearance of glyoxylate, determined by the method of Friedemann and Haugen[5]; thus, from 10 ml. of reaction mixture in a boiling-tube at 30° C., 15–20 μmoles of glyoxylate were lost in 10 min. in the presence of 1 ml. of fresh extract. After reaction, the solution contained a compound which formed a derivative with 2 : 4-dinitrophenylhydrazine giving a purple colour with 5 *N* sodium hydroxide; the absorption spectrum showed a maximum at 565 mμ and appeared identical with that of the *bis*-2 : 4-dinitrophenylhydrazone of glycolaldehyde (and glyoxal). The product of glyoxylate decarboxylation reacted with naphtharesorcinol in 23 *N* sulphuric acid to give a green colour; the absorption spectrum was similar to that recorded by Dickens and Williamson[6] for hydroxypyruvate and glycolaldehyde; and calculations based on the extinction coefficient at 660 mμ showed that one mole of glycolaldehyde would be formed from two moles of glyoxylic acid.

By analogy with the reactions[7] for the conversion of pyruvate to acetoin, with β-acetolactic acid as intermediate, the decomposition of glyoxylate may be formulated as follows:



We have not yet succeeded in separating these two enzymes, and our evidence, which is discussed below, for an intermediate step in the formation of glycolaldehyde is therefore indirect.

When extracts (5 ml.) were dialysed against phosphate buffer (4 lit.) for 24 hr. with stirring decarboxylation of glyoxylic acid was catalysed only when thiamine pyrophosphate and magnesium ions (each 1·5 μmole per ml.) were added with extract. Crude extracts catalysed the rapid oxidation of reduced diphosphopyridine nucleotide in the presence of glyoxylate, but this occurred only very slowly after dialysis. When thiamine pyrophosphate and magnesium were added, rapid oxidation of reduced diphosphopyridine nucleotide was restored, as shown in Fig. 1; however, this oxidation was not catalysed when glycolaldehyde was substituted for glyoxylate. Accordingly, it appears that the immediate product of glyoxylate decarboxylation, which we suggest is tartronic semialdehyde, is the acceptor of hydrogen from reduced diphosphopyridine nucleotide; the reduction product would be glyceric acid, although firm identification is hindered by the competing reaction which produces glycolaldehyde. Oxidation of reduced diphosphopyridine nucleotide in the presence of extract was much more rapid with presumed tartronic semialdehyde present than with hydroxypyruvate, which has also been suggested as a product of glyoxylate decarboxylation by *E. coli*[8].

Since it serves as a substrate for transketolase[9], the production of glycolaldehyde by the reactions described may be of significance in relation to carbohydrate synthesis by this organism; but since glycolaldehyde is itself a C_2-compound, *net* synthesis of cell constituents must be accomplished by a reaction sequence such as that proposed by Kornberg and Gotto (first communication), in which pyruvate malate and other tricarboxylic acid cycle compounds are formed. Support for a reaction sequence involving pyruvic acid was given by experiments with whole cells. When harvested from a glycine medium after

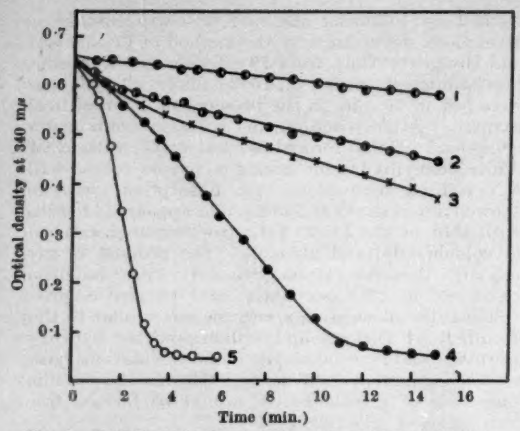


Fig. 1. Oxidation of reduced diphosphopyridine nucleotide by dialysed extracts of '*Pseudomonas A*' grown with glycine. The reaction was started by addition of 0·1 ml. of extract and followed by the fall in optical density at 340 mμ in mixtures made up to a total volume of 3·3 ml. with phosphate buffer (0·015 *M*, pH 7·2). Where stated, the following were added: reduced diphosphopyridine nucleotide, about 0·36 μmole; glyoxylic acid, 10 μmoles; thiamine pyrophosphate, 4 μmoles; magnesium sulphate, 5 μmoles. (1) Thiamine pyrophosphate and magnesium ions; no glyoxylate; (2) glyoxylate only; (3) glyoxylate and magnesium ions; (4) glyoxylate and thiamine pyrophosphate; (5) complete reaction mixture of glyoxylate, thiamine pyrophosphate and magnesium ions

about 18 hr. in the stationary phase, a lag in growth of 4 hr. occurred when suspensions were aerated in fresh glycine + mineral salts medium. During this time a keto-acid accumulated of which the spectrum of the 2 : 4-dinitrophenylhydrazone in alkali was identical with that of pyruvic acid; and when cell division began, pyruvate disappeared from the medium. An alternative reaction sequence for pyruvate production from glycine would be by conversion to serine and deamination of the latter; however, extracts from cells grown with glycine contained no serine dehydrase.

We are indebted to the Agricultural Research Council for a studentship to one of us (for A. G. C.).

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Formation of Glycerate from Oxalate by *Pseudomonas oxalaticus* (OXI) grown in Oxalate

An organism growing on oxalate as sole source of carbon and of energy must possess biosynthetic pathways capable of transforming this compound into all its various cell constituents. It has been suggested[1] that it might do this by oxidizing the oxalate to carbon dioxide, followed by fixation of the latter in a similar manner to that of a chemosynthetic autotroph[2]. However, it has recently been found[3] that a key enzyme of the carbon dioxide fixation cycle of autotrophs, carboxydismutase, is not synthesized by *Ps. oxalaticus* during growth on oxalate; also, carbon dioxide is not appreciably incorporated into phosphorylated compounds by such cultures. These findings eliminate the operation of an autotrophic type of metabolism and suggest that biosynthesis of cell constituents from oxalate conforms with that occurring with C_2-compounds other than acetate (see first communication). The present communication presents results indicating that incorporation of oxalate follows a similar route to that suggested for other pseudomonad species growing on glycollate (first communication) or glycine (second communication). The essential difference lies in the reduction of oxalate to glyoxylate, the latter compound being the first common intermediary metabolite of these three growth substrates.

Growing cultures of *Ps. oxalaticus* were incubated with [^{14}C] oxalate and the course of entry of the isotopic carbon into the cells was followed by chromatographic and radioautographic techniques already described[4,5]. The large amount of residual radioactive oxalate, which obscured the area of the chromatogram in which many phosphorylated compounds are found, was removed by incubating the aqueous eluates of such areas with oxalate decarboxylase obtained from *Collybia velutipes*[6]. This enzyme specifically decarboxylates oxalate to formate and carbon dioxide, both of which were easily removed on further chromatography. The results of this analysis are shown in Fig. 1, the percentages of the total radioactivity on each chromatogram that were contained in the separated compounds being plotted as functions of time. The only labelled compounds formed in 3 sec. and isolated under these conditions were glycine and phosphoglycerate. During the incubation period of 3–10 sec., the percentage of radioactivity contained in glycine diminished, while that in phosphoglycerate increased. This shows that isotope from oxalate enters glycine first and then phosphoglycerate, other metabolites such as sugar phosphates and alanine become labelled later. The following work with cell-free extracts indicates the possible nature of these initial transformations.

Crude, cell-free extracts prepared from the organism catalyse a rapid anaerobic decarboxylation of glyoxylate in which one mole of carbon dioxide is formed from two of glyoxylate. Using [^{14}C] glyoxylate

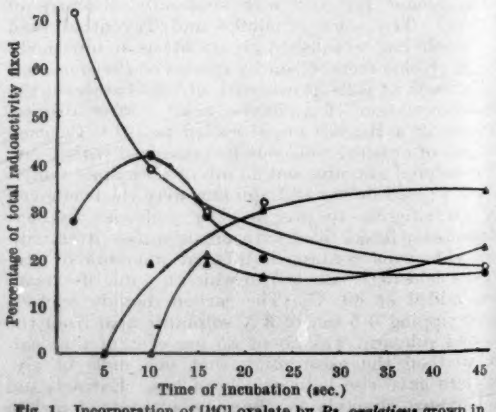

Fig. 1. Incorporation of [^{14}C] oxalate by *Ps. oxalaticus* grown in oxalate. Percentage of the total radioactivity present on each chromatogram that was contained in the separated compounds, plotted against time of incubation: ○—○, glycine; ●—●, phosphoglycerate; △—△, alanine; ▲—▲, sugar phosphates

(10^4 counts/min., under the conditions of radioassay used), and analysing the reaction products by chromatography and radioautography, the formation of radioactive glycerate (2,380 counts/min.) has been found after 15 min. anaerobic incubation, the yield of glycerate was increased almost three-fold by the addition of reduced diphosphopyridine nucleotide. No other radioactive product was found. These reactions are similar to those found in the pseudomonads grown in glycollate (first communication) and postulated in pseudomonads grown in glycine (second communication), and demonstrate a reductive conversion of glyoxylate to glycerate. The formation of some glycerate in the absence of added reduced diphosphopyridine nucleotide is probably due to endogenous reduced diphosphopyridine nucleotide present under the anaerobic conditions.

Formation of [^{14}C] glycerate (710 counts/min) was also observed in these extracts from [^{14}C] oxalate (2×10^4 counts/min.) in the presence of acetyl coenzyme A and reduced diphosphopyridine nucleotide. No other product was found on the chromatograms. Addition of these co-factors separately resulted in little synthesis of glycerate (less than 160 counts/min.).

These results indicate that the following reactions may occur in the crude extract, under the test conditions :

(i) 2 oxalate + 2 acetyl CoA → 2 oxalyl CoA + 2 acetate ;

(ii) 2 oxalyl CoA + 2 $DPNH_2$ → 2 glyoxylate + 2 CoA + 2 DPN ;

(iii) 2 glyoxylate + $DPNH_2$ → glycerate + CO_2 + DPN

Reaction (i), involving an acetyl coenzyme A transphorase, would lead to oxalyl coenzyme A, evidence for the existence of which has previously been presented[7].

The reduction of an acyl coenzyme A grouping to an aldehyde grouping, as suggested in reaction ii, has already been demonstrated[8] in extracts of *E. coli* with acetyl coenzyme A :

acetyl CoA'_2 + $DPNH_2$ → acetaldehyde + CoA + DPN

The overall effect of the reactions would be reductively to condense oxalate to glycerate and carbon dioxide.

Under the conditions of chromatography used in the whole-cell experiments, the presence of glyoxylate, the first labelled compound to be made from the activated oxalate in reaction ii, would not be observed. However, transamination reactions leading to [^{14}C] glycine formation would reflect the presence of [^{14}C] glyoxylate. The behaviour of tartronic acid semialdehyde (the postulated intermediate (preceding communications) in reaction iii) under the conditions of chromatography are not known. The presence of a glyceric acid kinase, as in the glycollate-grown *Pseudomonas ovalis* Chester (see first communication), could lead to the early formation of radioactive phosphoglycerate from glycerate, the product of reaction iii. The appearance thereafter of isotope in sugar phosphates, alanine and C_4-compounds could then be expected from established metabolic pathways.

Full details of this work will be published elsewhere.

We thank Sir Hans Krebs for his interest and encouragement. This work has been supported in part by the Rockefeller Foundation, and by the Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European Office, under Contract No. *AF* 61(052)–180.

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CONSTITUENTS OF HUMAN HÆMOGLOBIN

Separation of the Peptide Chains of Human Globin

SCHROEDER and his colleagues[1] reported that human hæmoglobin is composed of four polypeptide chains of two different kinds, now referred to as α- and β-chains. Since human hæmoglobin has two identical half-molecules, each half-molecule contains one α- and one β-chain. It was of interest to separate these two different polypeptide chains and to find the distribution between them of tryptic peptides 4, 23 and 26 (ref. 2) which contain the changes in amino-acid sequence associated with the abnormal human hæmoglobins S (ref. 3) and C (ref. 4), D_α (ref. 5) and I (ref. 6), and D_β (ref. 5) and E (ref. 7), respectively (Table 3).

Itano and Singer have shown[8] that human hæmoglobin splits into two *unequal* half-molecules during dissociation at *p*H 4·3. Presumably, there are units in the hæmoglobin molecule which can be represented by αα and ββ. By an ingenious experiment using this type of reversible dissociation, Vinograd, Hutchinson and Schroeder[9] were able to show that in a mixture of hæmoglobins A and S, the αα half-molecule is interchangeable, whereas the ββ one is not. These authors argued that the β-chain in hæmoglobin S is therefore different and that it carries the mutational change associated with hæmoglobin S. Their findings are confirmed by the experiments to be presented in this article, in the course of which the α- and β-chains themselves were prepared and examined.

The splitting of horse hæmoglobin was well demonstrated by Reichmann and Colvin[10], who calculated from physical studies that the molecule is composed of four chains, of two different kinds. By prolonged boundary electrophoresis at very low *p*H, these different polypeptide chains were separated. Indications of the presence of four sub-units had previously

been obtained by osmometry in strong salt solutions of both horse and human hæmoglobins[11]. In the present work, the initial experiments with human globin and hæmoglobin were modelled on Reichmann and Colvin's procedure. Whereas their method gave us results with horse globin which were similar to those published by these authors, all attempts failed to obtain indications of splitting of the human globin at low *p*H values. Further attempts were based on the experiences of Brand and Johnson[12], who were able to dissociate the seed protein legumin into about six similar sub-units by the action at alkaline *p*H of sodium dodecylsulphate.

The first positive indications that human hæmoglobin could be broken down to single chains came when human hæmoglobin A treated with sodium dodecylsulphate was examined in the ultracentrifuge at room temperature and *p*H 8·6. A single, symmetrical peak was seen, but the uncorrected sedimentation constant of $2{\cdot}0 \times 10^{-13}$ agreed reasonably well with a constant for sperm whale myoglobin (molecular weight 17,000) of $2{\cdot}3 \times 10^{-13}$ (uncorrected) obtained under identical conditions. These findings indicated that under these conditions such a hæmoglobin molecule was breaking down into quarter units, that is, into four polypeptide chains, the number expected from the analysis of the N-terminal amino-acids[1]. The corrected sedimentation constants (Table 1) are rather higher than might have been expected for quarter sub-units, no doubt due to the combination with rather large amounts of sodium dodecylsulphate. Boundary electrophoresis at 0·9° C. (Fig. 1) revealed two well-defined components in the ascending limb, although the boundaries on the descending side were not so well resolved. The final composition of this solution was: protein, 2·2 per cent; veronal, 0·12 *M*; sodium dodecylsulphate, 0·026 *M*; 0·36 gm. of sodium dodecylsulphate per gm. protein. This solution was dialysed in the cold against 43 volumes of 0·12 *M* veronal buffer, *p*H 8·6, overnight before electrophoresis.

Boundary electrophoresis in the Perkin–Elmer model 38*A* apparatus with continuous compensation produced milligram quantities of one of the two components as shown in Fig. 1. After some 24 hr. the 'fast' component or chain was removed from the cell and dialysed exhaustively against cold water before lyophilization.

Such a separation was quite reproducible, although there are reasons for believing that the exact proportion of sodium dodecylsulphate is critical. The method is also effective when hæmoglobin itself is used. Here, however, the two chains have slightly

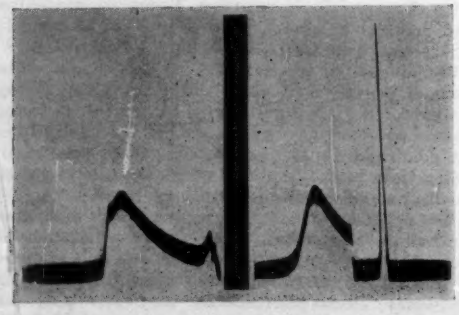

Fig. 1. Electrophoresis of human globin with sodium dodecylsulphate in veronal buffer, *p*H 8·6, ionic strength 0·12. Ascending limb on the right

Table 1. Behaviour of Globin and Hæmoglobin in the Presence of Sodium Dodecylsulphate

	Globin–sodium dodecylsulphate	Hæmoglobin–sodium dodecylsulphate
Electrophoretic mobilities		
'Fast' chain (β)	$10{\cdot}7 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ V.$^{-1}$	$12{\cdot}7 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ V.$^{-1}$
'Slow' chain (α)	$9{\cdot}6 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ V.$^{-1}$	$11{\cdot}3 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ V.$^{-1}$
Sedimentation constant (corrected S_{20})	$2{\cdot}40 \times 10^{-13}$	$2{\cdot}20 \times 10^{-13}$

Protein concentration = 2·2 per cent.

higher mobilities (see Table 1) due no doubt to the additional negative charges of the hæm groups.

Samples of the 'fast' chain have been subjected to tryptic digestion by the methods previously described[2], although with the help of a Jacobsen-Leonis *p*H-stat[13]. Comparison of fingerprints from such digests with those of ordinary hæmoglobin digests and of digests of globin which had been treated with sodium dodecylsulphate and dialysed, showed that approximately half the usual tryptic peptides were absent. This was confirmed by the examination of tryptic peptides using one-dimensional paper electrophoresis at *p*H 6·4 (ref. 2) followed by development with ninhydrin and with reagents specific for the amino-acids histidine, tyrosine, arginine and tryptophan[14]. There was a little doubt about the presence or absence in the 'fast' chain of a few peptides, but it was clear that peptide 26 (hæmoglobin E and D_β changes) is present, whereas peptide 23 (hæmoglobin D_α and I changes) is absent and must be on the 'slow' chain. Peptide 4 (hæmoglobin S and C changes) is present, as was shown by electrophoresis at *p*H 3·6 of the band of neutral peptides obtained at *p*H 6·4, followed by a qualitative analysis of the amino-acid composition of the peptide containing histidine. Hence peptides 4 and 26 are on the 'fast' chain and peptide 23 is presumably on the 'slow' chain.

These findings confirm Schroeder's conclusion about the presence of two types of polypeptide chain in human hæmoglobin. Further experiments showed that the 'fast' chain is the β-chain, and not the α-chain as provisionally indicated[15].

In order to obtain a clearer picture of the distribution of the tryptic peptides between the α- and the β-chain, it was clearly desirable to prepare both chains and to identify them. Wilson and Smith[16] have worked out three ways of obtaining pure samples of the two different polypeptide chains of horse globin. They use preparative boundary electrophoresis at low *p*H, fractional precipitation with acid/acetone and ion exchange chromatography in a urea gradient. The last method was used, without modification, in the present experiments with globin from human hæmoglobin A. Whole globin was fractionated on an 'Amberlite IRC-50/XE 64' or 'CG 50' type 2 column with a urea gradient[16] of 2 *M*–8 *M*, always adjusted to *p*H 1·9 with hydrochloric acid. As Fig. 2 shows, two peaks are obtained, representing the α- and the β-chain, in that order, although the separation is poorer than that obtained by Wilson and Smith for horse globin. It was to be expected from their behaviour in boundary electrophoresis that the human globin chains would be more difficult to separate on an ion-exchange column. No doubt the method could be adapted to suit human globin better than it does at present. Wilson and Smith found three peaks in the case of horse globin, peaks 2 and 3 both having the same N-terminal dipeptide, namely, valyl-glutamyl-. The reason for the doubled peaks could be association of the molecules of this chain—shown by these chains in

solution—or it could be connected with the fact that horse hæmoglobin usually shows two electrophoretic components. If the latter explanation be correct, then this electrophoretic difference can be assumed to reside in the polypeptide chain of horse globin which begins with valyl-glutamyl-. Since all carboxyl groups are uncharged at such a low *p*H, the difference would probably involve the basic amino-acids of this chain.

The chains were obtained by dialysis and freeze drying. In part they were digested with trypsin after heat denaturation. Examination of such digests by fingerprinting[2] and by one-dimensional ionophoresis at *p*H 6·4[3] showed that the known tryptic peptides are fairly evenly distributed between the two fractions (Fig. 3). The investigation of some of these peptides is still proceeding. By the methods used in the investigation of the human hæmoglobins 'D' (ref. 5) —particularly by specific staining reactions—it was possible to show that peptide 23 and peptide 26 occurred in the fractions I and II, respectively. This is interesting, because peptide 23 is involved in the mutations which result in hæmoglobins I and D_a and peptide 26 in hæmoglobins E and D_β. The location of peptide 4 (hæmoglobins S and C mutations) was not immediately apparent, because in hæmoglobin A this peptide is one of several uncharged peptides at the *p*H used for the ionophoresis, namely, 6·4. However, when the neutral peptides from digests of the two chains were compared by ionophoresis at *p*H 3·6, it became clear that peptide 4 is part of the chain in fraction II, namely, the β-chain (Fig. 3). This is in agreement with Vinograd and Schroeder's findings[9].

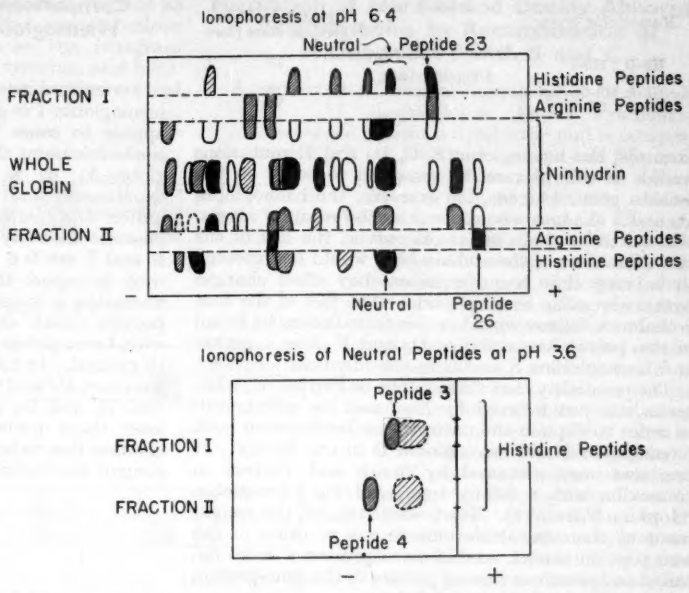

Fig. 3. Tracings of ionograms of tryptic digests; ionophoresis at *p*H 6·4. Colour reactions for specific amino-acids as in ref. 14

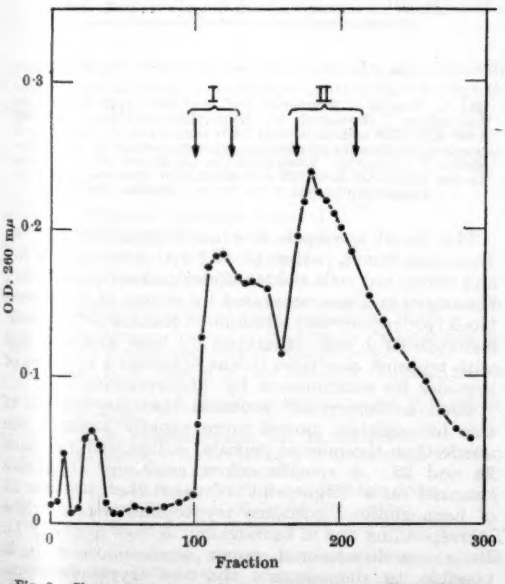

Fig. 2. Chromatography of human globin in a gradient of urea (see text for details)

In order to identify fractions I and II with Schroeder's α- and β-chain, each fraction as well as whole globin were subjected to degradation from the amino-end with Fraenkel–Conrat's paper strip modification[17] of the Edman method. Table 2 shows that fraction I begins with valyl-leucine and is therefore the α-chain, whereas II begins with valyl-histidine- and is the β-chain. Table 3 indicates the distribution of the peptides carrying the various mutational alterations.

These results have interesting genetic implications[18]. The findings of the amino-acid changes in hæmoglobins S, C and E support the widely accepted hypothesis of a correlation between the linear fine-structure of the polypeptide chain controlled by that gene. When discussing a protein such as human hæmoglobin, which has two kinds of peptide chains, one is tempted to extend these ideas in the following way: suppose that there are two hæmoglobin genes; an 'α-chain gene' to control the amino-acid sequence of the α-peptide chain, and a 'β-chain gene'. Mutations may affect one or the other of these genes; for

Table 2

Schroeder's nomenclature of chains (ref. 1)	Whole globin*	Fraction I	Fraction II
	α + β	α	β
Edman degradation (ref. 17)			
Step 1	Valine + + +	Valine + +	Valine + +
Step 2	Leucine + +	Leucine + +	Leucine trace
(acid extract)	Histidine +	Histidine trace	Histidine +
(basic extract)	Histidine + +	Histidine trace	Histidine + +
N-terminal sequence	val-leu-val-his-	val-leu	val-his

* Double quantity.

Table 3

Hæmoglobin types		Peptide in which change takes place
	α-Peptide chain	
Hb-D_α, Hb-I		23
	β-Peptide chain	
Hb-S, Hb-C		4
Hb-D_β, Hb-E		26

example, the hæmoglobin S, C, D_β and E mutations would, in this picture, be expected to occur on the β-chain gene, whereas, for example, the hæmoglobin D_α and I changes would occur as the result of alteration on the α-chain gene. Moreover, the loci of the two mutations of the α-chain gene would be expected to be very close together, since they effect changes within the same small peptide. The loci of the four β-chain mutations would in this formulation be found in two pairs—hæmoglobins D_β and E close together and hæmoglobins S and C in the identical location[4].

The possibility that there might be two hæmoglobin genes was put forward by Neel and his colleagues[18] in order to explain the pattern of inheritance of both hæmoglobin S and hæmoglobin G in one family. It has also been proposed by Smith and Torbert in connexion with a family tree involving hæmoglobin 'Hopkins 2' (ref. 19). The present results, the assignment of chemical alterations to one or other of the two peptide chains, enable us to present a more detailed and more convincing picture of the gene-protein relationship in the hæmoglobins.

I wish to thank Dr. D. B. Smith and Dr. J. Vinograd for their courtesy in allowing me to see copies of their papers prior to publication. Thanks are also due to Dr. P. Johnson, of Cambridge, for many helpful discussions. The expert assistance of Mrs. R. Fishpool and Miss E. Sullivan is gratefully acknowledged, as is the help given by Mr. Boon, of the Biochemical Laboratory, Cambridge, who carried out the ultracentrifuge determinations. This work was supported in part by a grant from the Medical Foundation, Inc., Boston, the help of which is gratefully acknowledged.

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Comparison of Normal Adult Human Hæmoglobin with Hæmoglobin I by 'Fingerprinting'

According to Rucknagel *et al.*[1] the occurrence of hæmoglobin I is genetically determined but does not appear to cause a severe anæmia. It has a lower isoelectric point than the normal hæmoglobin (hæmoglobin-A) in a moving-boundary electrophoresis experiment, and migrates more rapidly in alkaline buffers and less rapidly in acid buffers; the electrophoretic mobility differences[2] between hæmoglobins A and I are 0·6 to 0·7 × 10^{-5} cm^2. $sec.^{-1}V.^{-1}$. We wish to report that in hæmoglobin I a mutational alteration is found in tryptic peptide 23 (ref. 3), the peptide which also contains the change[4] associated with hæmoglobin D_α. The other peptides appear to be normal. In hæmoglobins S and C, tryptic peptide 4 is altered[5,6] and tryptic peptide 26 in hæmoglobins E (ref. 7), and D_β (ref. 4). Therefore, we know of at least three parts of the molecule which undergo changes due to mutation in the gene or genes which control the biosynthesis of the hæmoglobin molecule.

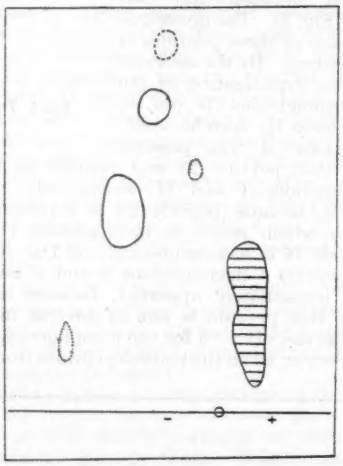

Fig. 1. Tracing of 'fingerprint' (ref. 3) of the tryptic digest from hæmoglobn I developed for tryptophan-containing peptides (ref. 10). The area of peptide 23 is cross-hatched, indicating a positive reaction for tryptophan, whereas peptide 23 of hæmoglobin A is negative. Electrophoresis was carried out at *p*H 6·4 in the horizontal direction and subsequent chromatography in butanol/acetic acid in the vertical direction (ref. 3)

The blood specimen was made available by Dr. Tocantins from a patient (J. W.) with hæmoglobin A-I and whose red cells sickled under certain conditions[8]. Hæmoglobin I was separated by means of the starch block electrophoresis technique of Kunkel[9]. 'Purified' hæmoglobin I was denatured by heat and digested with trypsin[3], and from it was obtained a mixture of peptides for examination by 'fingerprinting'.

Such a 'fingerprint' revealed that peptide 23 of this hæmoglobin moved more rapidly towards the anode than the normal peptide, in line with peptides 24 and 25. A specific colour reaction[10] (Ehrlich's reagent) on a 'fingerprint' revealed that peptide 23 of hæmoglobin I contains tryptophan whereas the corresponding one in hæmoglobin A does not (Fig. 1). In a one-dimensional paper electrophoresis it is possible to demonstrate the new tryptophan-containing peptide under an ultra-violet lamp. It is

not yet clear whether other amino-acids in peptide 23 are involved in the change. The specific colour reactions[10] carried out directly on the ionogram revealed the presence of arginine, tyrosine, and histidine (Fig. 2) which also occur in peptide 23 of normal

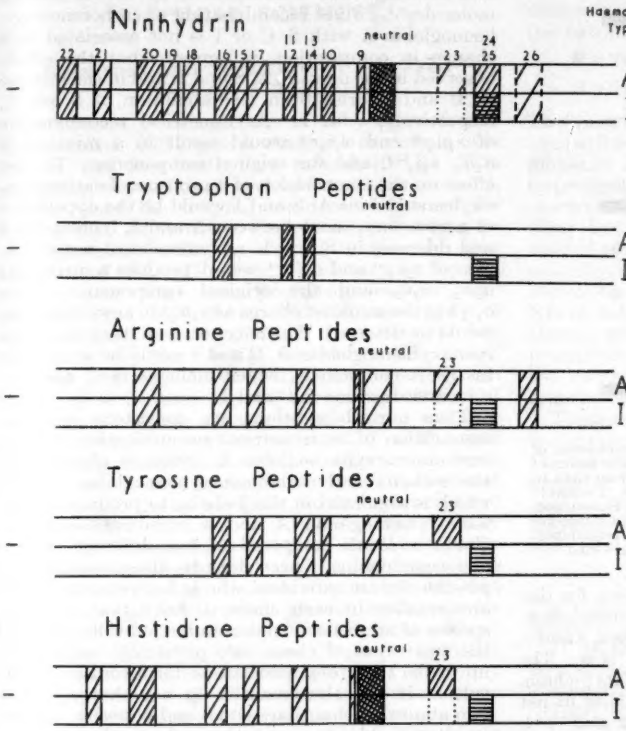

Fig. 2. Comparison by one-dimensional paper electrophoresis (ref. 3) of tryptic digests of hæmoglobins A and I at *p*H 6·4, followed by colour reactions for specific amino-acids (ref. 10). Note that peptide 23 of hæmoglobin I is in alignment with peptides 24 and 25 of hæmoglobin A

hæmoglobin. It is hoped that further experiments will reveal in detail the change undergone by peptide 23.

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Formation of Normal and Doubly Abnormal Hæmoglobins by Recombination of Hæmoglobin I with S and C

A MOLECULE of normal human adult hæmoglobin (hæmoglobin A) is symmetrical about a dyad axis[1] and is composed of four hæm groups and four polypeptide chains, a pair of α-chains and a pair of β-chains[2]. Hæmoglobins A, S, and C dissociate asymmetrically in acid into two unlike sub-units, each of which consists of a pair of identical chains and two hæms[3,4]. Complete molecules are regenerated from these sub-units when an acidified solution is neutralized, and sub-units are exchanged when a mixture of a pair of unlike hæmoglobins is dissociated and recombined. The chemical abnormalities of hæmoglobins S and C occur at the same place in the β-chain[5,6]. Hæmoglobin I, on the other hand, is abnormal in the α-chain[7]. We wish to report evidence for the formation of hæmoglobin A and a doubly abnormal molecule when hæmoglobin I is recombined with hæmoglobin S or C.

A mixture of hæmoglobins A and I received from Dr. L. M. Tocantins was used[8]. The mixture, and mixtures of it with hæmoglobin S and with hæmoglobin C, were examined electrophoretically both before and after recombination. All samples were in the form of carbonmonoxyhæmoglobin. Recombined mixtures were prepared by dissociation in acetate-sodium chloride buffer of *p*H 4·7, Γ/2 0·2 (ref. 4) for 4 hr. at 5° C. followed by dialysis against potassium phosphate buffer of *p*H 6·3, Γ/2 0·02, for about 20 hr. at 5° C. The resulting samples were analysed by moving-boundary electrophoresis in the same buffer for 5 hr. at 3° C. with a potential gradient of 8·5 V. cm.$^{-1}$. Control electrophoretic analyses on undissociated aliquots of the same mixtures were carried out under identical conditions. Satisfactory resolution was obtained only in the ascending limb. When a mixture of carbonmonoxyhæmoglobins A, I and S was recombined, a new component appeared between A and I. At the same time the proportion of hæmoglobin A increased and the proportions of I and S decreased. Recombination of carbonmonoxyhæmoglobins A, I and C produced no new electrophoretic component; however, the proportion of hæmoglobin A increased with decrease of I and C. No new component or change in proportions occurred when carbonmonoxyhæmoglobins A and I were recombined. The results are shown in Fig. 1 and Table 1. The numerical values of Table 1 are directly proportional to the areas under the peaks of Fig. 1. These areas are probably anomalous because of low ionic strength of buffer. However, since aliquots of the same mixture were used in each pair of analyses, change in relative areas following recombination must reflect change in true composition.

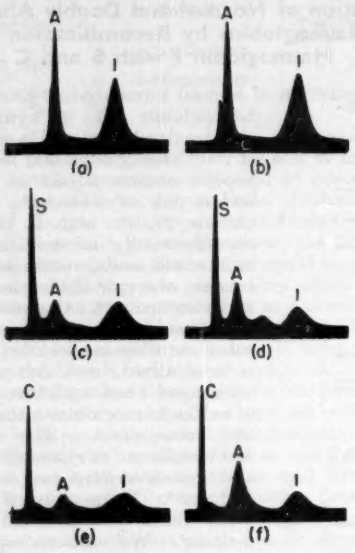

Fig. 1. Ascending boundary patterns from electrophoresis of 1·0 per cent carbonmonoxyhæmoglobin in phosphate buffer of pH 6·3, Γ/2 = 0·02. The direction of migration is from right to left. From left to right the components are: (*a*) A, I control; (*b*) A, I recombined; (*c*) S, A, I control; (*d*) S, A, I recombined. Note the new component ($s_2i_2^{-2}$) between A and I and the increase in proportion of A; (*e*) C, A, I control; (*f*) C, A, I recombined. Note the increase in the proportion of A ($a_2u_2 + c_2i_2$)

A simplified nomenclature[3,4] is convenient for discussion of dissociation-recombination phenomena. The β-chains of hæmoglobins A, S and C are *a*, *s* and *c*, respectively; and their common α-chain is *u*. The abnormal α-chain of hæmoglobin I is *i*, and its β-chain, presumably normal, is *a*. The abnormalities in net charge of hæmoglobins S and C are + 2 and + 4, respectively[5]. The mobility of hæmoglobin I indicates an abnormality in net charge of close to − 4. The net charge of the unaltered portions of the molecules may be omitted in the present discussion; accordingly carbonmonoxyhæmoglobins A, S, C and I may be written a_2u_2, $s_2u_2^{+2}$, $c_2u_2^{+4}$, and $a_2i_2^{-4}$, respectively. Asymmetric dissociation would result in the sub-units a_2, s_2^{+2}, c_2^{+4}, u_2, and i_2^{-4}.

We can now interpret our results with the assumption of asymmetrical dissociation and recombination. When a mixture of a_2u_2 and $a_2i_2^{-4}$ is recombined, exchange of a_2 sub-units can occur without effect on electrophoretic composition, in agreement with our observations on the mixture of carbonmonoxyhæmoglobins A and I. The situation is analogous to the exchange of u_2 sub-units when mixtures of carbonmonoxyhæmoglobins A, S and C were recombined. Recombined mixtures yielded the same electrophoretic patterns as corresponding untreated mixtures, and exchange was demonstrated by use of labelled molecules[3,4]. Since recombination of carbonmonoxyhæmoglobin A with S, C or I is not associated with change in composition, it appears that the effects observed in the mixture A, S and I, and in the mixture A, C and I, arise from recombination of S and C, respectively, with I. Asymmetrical recombination of $s_2u_2^{+2}$ and $a_2i_2^{-4}$ would result in a mixture of a_2u_2, $s_2i_2^{-2}$, and the original components. The net effect on the composition of a mixture of carbonmonoxyhæmoglobins A, S and I would be the appearance of a new component between A and I, increase in A and decrease in S and I. Asymmetrical recombination of $c_2u_2^{+4}$ and $a_2i_2^{-4}$ would produce a mixture of a_2u_2, c_2i_2, and the original components. Since c_2i_2 has the same net charge as a_2u_2, no new component would be detected. The effect on a mixture of carbonmonoxyhæmoglobins A, C and I would be an increase in the A component, which includes both a_2u_2 and c_2i_2, and decrease in C and I.

Thus our observations are consistent with the assumption of asymmetrical recombination between carbonmonoxyhæmoglobin I, which is abnormal in the α-chain, and carbomonoxyhæmoglobin S or C, which is abnormal in the β-chain, to produce carbonmonoxyhæmoglobin A and a carbonmonoxyhæmoglobin molecule composed of two different pairs of abnormal chains. According to these results, it is possible for an individual who is heterozygous for an abnormality in each chain to have four molecular species of adult hæmoglobin in his red cells, provided the two types of chains are physically independent up to the time they associate to form complete molecules. If the abnormalities in net charge of the two abnormal chains are equal and opposite, the four species would appear as three electrophoretic components. In either circumstance one of the four species would be composed entirely of normal chains. Formation of a normal protein molecule by association of the normal components of two different abnormal molecules suggests one possible mechanism for extranuclear formation of a normal enzyme molecule by the co-operative action of two mutant strains of the same organism, neither of which produces active enzyme[4].

Table 1. Composition of Hæmoglobin Mixtures before and after Recombination

Mixture		Electrophoretic composition: per cent of total				
		$a_2i_2^{-4}$ (I)	$s_2i_2^{-2}$	a_2u_2 (A)	$s_2u_2^{+2}$ (S)	$c_2u_2^{+4}$ (C)
A+I	Control	53	—	47*	—	—
	Recombined	55	—	45*	—	—
A+I+S	Control	34	—	24*	42	—
	Recombined	23	11	32	34	—
A+I+C	Control†	36	—	20	—	44*
	Recombined	22	—	42‡	—	36*

* Includes 3–4 per cent of trailing shoulder.
† Re-run of original control; C peak too sharp in original for determination of area.
‡ Probably includes c_4i_2.

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LETTERS TO THE EDITORS

PHYSICAL SCIENCES

Spectrum and Structure of the Free Methylene Radical

SEVENTEEN years ago, one of us[1] suggested that the so-called λ4050 group observed in comets was due to the free methylene radical (CH_2). This suggestion was not confirmed by later experiments[2–4].

Ever since the original misidentification of the 4050 group, experiments have been proceeding in this laboratory with the aim of finding the true spectrum of CH_2. However, it was only at the end of last year that some promising features were observed near 1400 A. in the flash photolysis of diazomethane. Recently, one of these features has been positively identified as due to free CH_2.

In the first order of our 3-m. vacuum spectrograph the group of features under consideration consists of six slightly diffuse 'lines' which show, on substitution with deuterium, a marked shift, thus establishing that the carrier of this group must contain one or more hydrogen atoms. The strongest feature in this group occurs in the flash photolysis of ordinary diazomethane at 1414·5 A., in fully deuterated diazomethane at 1415·8 A. When partly deuterated diazomethane is used, a third feature is observed at 1415·5 A. In this way it is shown that there are two hydrogen atoms present in the molecule responsible for this spectrum. While this made it very likely that CH_2, CHD and CD_2 are responsible for the observed spectra, conclusive proof could only be obtained by a study of the rotational fine structure of some of the features observed.

Unfortunately, it turned out that in the fourth order of our vacuum spectrograph all the features observed in the flash photolysis of ordinary diazomethane were rather diffuse. However, in observing the corresponding spectrum in deuterated diazomethane, it was found that the principal feature at 1416 A. did show a rather well-resolved fine structure. This difference between the deuterated and non-deuterated molecule is very similar to the condition found[5] for the 2160 A. band of CH_2 and CD_2, and is apparently due to the fact that a deuterated molecule predissociates much less easily than a non-deuterated molecule.

The fine structure of the 1415·8 A. feature in the deuterated molecule consists of a simple *P*- and *R*-branch, each of which shows a clear alternation of intensity. The presence of this alternation proves beyond doubt that there must be two symmetrically placed identical atoms (deuterium atoms) in the molecule. The absence of a *Q*-branch proves that this is either a parallel band of a linear molecule or the $K = 0$ sub-band of a parallel band of a (nearly) symmetrical top molecule such that rotation about an axis perpendicular to the figure axis exchanges the deuterium atoms. Further confirmation was obtained by the observation of a similar band without an alternation of intensity in the flash photolysis of partly deuterated diazomethane. The spacing of the lines in the fine structure of the bands in the fully deuterated and partly deuterated compounds supplies values for the rotational constants (reciprocal moments of inertia) in the upper and lower states of the two molecules involved. These values are:

$$B'(CD_2) = 3{\cdot}59_5 \qquad B''(CD_2) = 3{\cdot}95_0 \text{ cm.}^{-1}$$
$$B'(CHD) = 4{\cdot}80_6 \qquad B''(CHD) = 5{\cdot}33_2 \text{ cm.}^{-1}$$

The magnitudes of these rotational constants are compatible only with the assumption that the molecule involved is of the type XD_2 and XHD respectively. From the method of production of the spectrum it appears impossible that X can be anything but C (carbon). The ground-state and first excited state of NH_2 are known, and definitely are not involved in the bands under investigation. Moreover, both the well-known spectra of ND_2 and ND in the visible and in the ultra-violet regions are absent, while the spectrum of CD (and CH) is very prominent. Thus we consider the identification of the carrier of the spectrum as free methylene as established.

The simplest and most obvious interpretation of the observed fine structure would appear to be that the molecule is linear in both the upper and lower states of the transition. With this assumption one obtains two independent values for the CH (or CD) distance from the rotational constants of B'' of CD_2 and CHD, namely:

$$r_0'' = 1{\cdot}029 \text{ and } 1{\cdot}034 \text{ A.}$$

The slight difference could be accounted for by the effects of zero-point vibration.

If, on the other hand, one assumes the molecule to be non-linear and uses the two moments of inertia to determine the two structural parameters r_0'' and $\angle$HCH one finds:

$$r_0'' = 1{\cdot}071 \text{ A. and } \angle HCH = 140°$$

However, these results imply that the rotational constant $A''(CD_2)$ would be 41·7 cm.$^{-1}$ and therefore several other sub-bands with $K = 1, 2, 3, \ldots$ would be expected, for which no evidence has been found. The absence of these sub-bands could be accounted for if it is assumed that the upper state is linear or that levels with $K \neq 0$ in the upper state are strongly predissociated and therefore escape observation. On account of the effect of zero-point vibration, a definite decision between the linear and non-linear structure of the molecule in the ground-state is not yet possible. All one can say definitely at this moment is that the HCH angle is between 140° and 180°.

The fact that in CD_2 the strong lines are those of odd J indicates that the lower electronic state of the transition is not totally symmetrical. Indeed, for linear CH_2 the predicted[6] ground-state is readily seen to be $^3\Sigma_g^-$, which would lead exactly to the observed intensity alternation. It is conceivable that the lower state of the transition, even though it has been observed in absorption, is not the ground-state of the molecule, but this would entail the assumption that the molecule can survive up to 5,000 collisions with the inert gas molecules present before going over to the real ground-state.

While the question of the ground-state still remains to be settled, we believe that it has been definitely established that the spectrum under consideration is due to the free methylene radical and that in the

lower state of the observed transition the molecule has an HCH angle between 140° and 180° and a non-totally symmetrical electronic structure. The life-time of the CH_2 radical under the conditions of our experiment (partial pressure of diazomethane before photolysis 0·02 mm., partial pressure of inert gas 2 mm. or more) was about 15 μsec., that is, of the order of the time between collisions of CH_2 with CH_2N_2.

Many members of this laboratory have contributed to the final success of these investigations by their suggestions, critical advice and other help. To all of them we are greatly indebted.

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Removal of Dipolar Broadening of Nuclear Magnetic Resonance Spectra of Solids by Specimen Rotation

The nuclear magnetic resonance spectrum of a solid rotated at high speed consists of a narrowed central line and a set of side-spectra spaced at integral multiples of the rotation rate of either side[1,2]. The interaction energy between each pair on nuclear magnets in the solid contains the angular factor $(3\cos^2\theta - 1)$, where θ is the angle between the internuclear vector and the applied field. When the solid rotates about an axis making an angle α with the applied field, the angular factor may be expressed[3] as the sum of its mean value $\frac{1}{2}(3\cos^2\alpha - 1)(3\cos^2\gamma - 1)$, where γ is the angle between the internuclear vector and the axis of rotation, and two time-dependent terms, $(3/2)\sin 2\alpha \sin 2\gamma \cos \omega_r t$ and $(3/2)\sin^2\alpha \sin^2\gamma \cos 2\omega_r t$, where ω_r is the angular frequency of rotation. The two time-dependent terms lead to the formation of the side-spectra. It should be noted that when α is 90°, as in our earlier experiments[1,2], the first of these terms is absent, so that side-spectra occur in this case at even multiples of ω_r only; for general α, the side-spectra occur at odd and even multiples of ω_r. The form of the mean value of the angular factor for each nuclear pair leads to the conclusion that the central spectrum should have the shape found for the static crystal when the applied field is directed along the axis of rotation, but reduced in width by the factor $\frac{1}{2}(3\cos^2\alpha - 1)$, and having a second moment[4] reduced by a factor $\frac{1}{4}(3\cos^2\alpha - 1)^2$. Thus, in particular, when α has the value $\cos^{-1}(1/\sqrt{3}) = 54° 44'$, the dipolar broadening of the central line should be removed[5]. With a speed of rotation large compared with the static line-width, the side-spectra will be displaced far from the centre and will be weak, leaving only a sharp central line broadened by residual non-dipolar causes.

We have extended our experiments on a rotating crystal of sodium chloride to cover a range of angles α from 50° to 100°. The axis of rotation was [001]. The measured width of the central line of the sodium-23 resonance spectrum determined between derivative maximum and minimum is plotted in Fig. 1. Also shown in Fig. 1 is the curve of $\frac{1}{2}|3\cos^2\alpha - 1|\delta\nu_0$, where $\delta\nu_0$ is the line-width for the static crystal with the applied field along [001]. The general agreement is quite good, but it is to be noticed that when $\alpha = 54° 44'$, the central line has a finite width of 200 c./s., which is greater than the instrumental breadth. This residual width may in this crystal arise from interactions between the electric quadrupole moments of the sodium-23 nuclei and electric field gradients caused by imperfections in the cubic crystal. The central line thus has a residual width about one-tenth of that for the static crystal, and a second moment of the order of 1 per cent of that for the static crystal. Such broadening would be difficult to detect while it is overlaid by the much larger dipolar broadening of the static crystal.

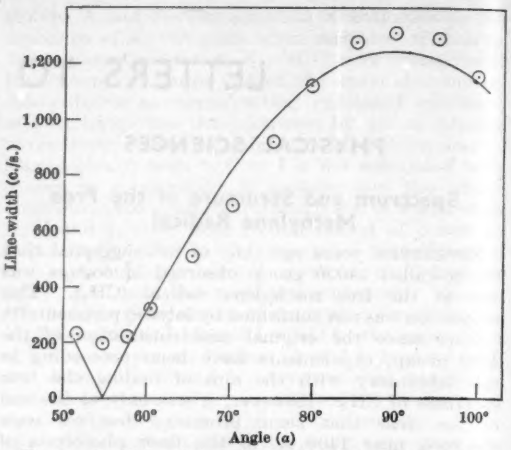

Fig. 1. Variation of line-width of the sodium-23 resonance spectrum of a rotating crystal of sodium chloride with the angle α between the axis of rotation and the direction of the applied field. The full line is the theoretical curve for a spectrum broadened solely by dipolar interactions; the circles are the experimental observations

In Fig. 2 is shown the derivative spectrum for $\alpha = 54° 44'$ at a rotation rate of 810 c./s. The first three side-spectra are visible on either side. Although the rate of rotation was sufficient to separate the side spectra from each other and from the central line, it was insufficient to remove them from the scene.

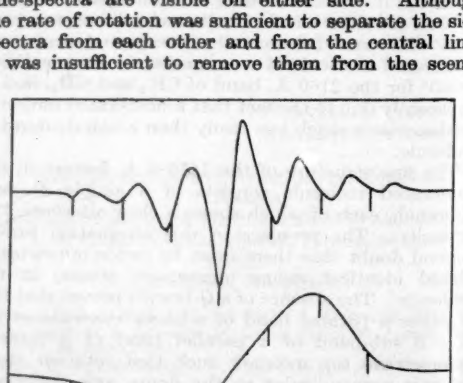

Fig. 2. The upper curve is the derivative spectrum for the crystal rotated at 810 c./s. about an axis making an angle 54° 44′ with the direction of the field. The lower curve is the derivative spectrum for the static crystal. The markers are 700 c./s. apart

Fig. 2 also shows, for comparison, a spectrum obtained when the crystal was at rest.

Several practical consequences appear to follow from the removal of dipolar broadening from the central line. In the first place, the position of the central resonance frequency can be determined with much greater precision; in our example the improvement is by a factor of ten. In this way a greater accuracy may be obtained in the measurement of nuclear magnetic moments when solid specimens are used. Secondly, the relative chemical shifts (and for metals, Knight shifts) may be determined with much greater precision. Thirdly, broadening or fine-structure caused, for example, by quadrupolar interactions, electron-coupled spin exchange or different chemical shifts on non-equivalent sites, may be revealed by the removal of the dipolar broadening which, in solids, usually masks them.

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Electron Microscopy of Oleophobic Monolayers

Oleophobic monolayers of stearic acid on mica have been studied by means of the electron microscope. Mica was chosen as the substrate because clean surfaces, which are molecularly flat over large areas, are easily obtained by cleavage. Stearic acid was retracted from a solution in hexadecane in the manner described by Bigelow, Pickett and Zisman[1] and a preshadowed carbon replica of the surface taken; 40 per cent gold/palladium was found to be the most suitable shadowing material.

Fig. 1 shows a typical result. The contrast of the micrograph is enhanced by agglomeration of the shadowing material on those parts of the surface covered by stearic acid. It will be seen that the monolayer is present in islands, which measurement of shadow-lengths show to be 25 A. thick. A close examination of the monolayer islands does in some instances show a tendency for the metal particles to arrange themselves in lines, which may suggest some regular array, within the islands, of the micelles which Epstein[2] suggests exist in monolayers.

Monolayers of stearic acid retracted from the melt[3] do not show the island structure of those retracted from hexadecane solution, so this structure cannot be attributed to evaporation of stearic acid during pumping prior to shadowing, or to bombardment of the surface by metal particles. It is evident from Fig. 1 that the surface is only partially covered by stearic acid, so it is pertinent to ask how it is that the surface is oleophobic. It is suggested that hexadecane is incorporated in the monolayer. The attractive forces between hydrocarbon chains are believed to be responsible for the orientation of long-chain polar compounds at both the solid and liquid interfaces; and Sobotka and Rosenberg[4] have shown that it is possible to spread mixed films of stearic acid

Fig. 1. Electron micrograph of a replica of a mica surface which has been dipped in a 0·1 per cent by weight solution of stearic acid in hexadecane for 30 sec. The arrow shows the direction of shadowing. The island structure of the monolayer is apparent

and long, straight-chain hydrocarbons on water surfaces, where the hydrocarbon will not spread by itself. A similar mechanism may operate in this case. Hexadecane is able to orient itself alongside adsorbed molecules of stearic acid, and may be gradually replaced as more stearic acid diffuses to the surface.

Although no quantitative results are available yet, it is evident from micrographs that the area covered increases with time of immersion, and with concentration of solution for a given time of immersion. Furthermore, if a monolayer is prepared by retraction of a drop on the mica surface, it is found that only a third of the surface is covered by acid. Fig. 2 shows a close-packed hexagonal arrangement of molecules where the black circles represent stearic acid and the white circles hexadecane. It can be seen that each hexadecane molecule has three stearic acid molecules among its six nearest neighbours. It is suggested that when a stearic acid molecule is adsorbed, hexadecane molecules are able to orient themselves beside it. When the condition in Fig. 2 is reached, the bulk liquid rests on an oleophobic surface of methyl groups and retracts, being unable to differentiate between the end groups of the acid and the hydrocarbon. Since the hexadecane has a much greater vapour pressure than stearic acid, it is pumped off during the preparation of the replica, and then the acid molecules collapse into islands as shown in Fig. 1. In no case has an oleophobic film been found to cover significantly less than a third of the surface.

The observations of Bigelow and Brockway[5], who find only a small reduction in contact angle when the

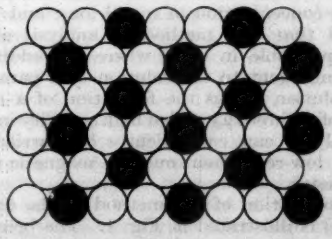

Fig. 2. Schematic representation of stearic acid and hexadecane molecules in a monolayer containing the minimum number of stearic acid molecules necessary for the surface to be oleophobic. The black circles represent stearic acid and the white hexadecane

density of adsorbed acid molecules is reduced by dipping in hexadecane, may be explained as a result of the reverse process taking place, hexadecane replacing stearic acid which has been dissolved away. These authors do point out that a surface may become oleophobic before the adsorption process is complete.

Identical observations have been made on retracted monolayers of long-chain aliphatic alcohols and primary amines. It has not been possible to observe any island structure when the mica has not been immersed in solution long enough for it to become oleophobic, although Bigelow and Brockway were able to obtain faint layer lines in electron diffraction pictures. It may well be that any clusters of molecules oriented on the surface are too small to be replicated satisfactorily.

I am indebted to Dr. F. P. Bowden and Dr. D. Tabor of this Laboratory for helpful discussion, and to Joseph Lucas, Ltd., for a research grant.

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Magnetometric Titration: a New Method of Chemical Analysis

IT is well known that the addition of paramagnetic solutes to water brings about a marked reduction in the nuclear spin-lattice relaxation time of solvent protons[1], the inverse of the relaxation time being linearly related to solute concentration according to the equation:

$$(T_1^{-1} - T_0^{-1}) = \alpha\mu_N^2 C$$

where T_1 and T_0 are spin-lattice relaxation times in the presence and absence of added solute, respectively; α is a proportionality constant; μ_N is a parameter expressing the effectiveness of a particular solute in this phenomenon, sometimes called the 'effective' paramagnetic moment of the solute; C is the concentration of the solute. In the case of solutions of paramagnetic complex ions, the magnitude of the effect (that is, the value of μ_N) depends on the nature of the co-ordinated groups[2]. We find that the phenomenon enables the replacement of one ligand by another in solution to be followed with sufficient precision to form the basis for a method of determining the concentration of metal ions, and it is now suggested that this method of analysis should be widely applicable in cases where the addition of a complexing agent to the solution of a paramagnetic ion in solution causes the formation of a new complex ion of different μ_N and of high stability in solution. The analyses can conveniently be carried out by use of a low-resolution nuclear magnetic-resonance spectrometer.

The application of the method to the titration of nickel(II) is illustrated in Fig. 1. The breaks in the curve correspond to equivalence points, in a manner somewhat reminiscent of conductimetric titrations. Nickel(II) represents a particularly favourable and

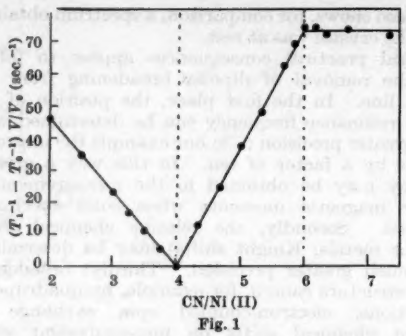

Fig. 1

interesting case for this method, since the diamagnetic species $[Ni(CN)_4]^{2-}$ does not catalyse the spin-lattice relaxation at all. Values of $(T_1^{-1} - T_0^{-1})$, corrected for the dilution effect which accompanies addition of titrating agent, are plotted against the amount of potassium cyanide added to an ammoniacal solution of nickel cyanide, that is, a solution of $[Ni(NH_3)_6]^{2+}$ $[Ni(CN)_4]^{2-}$.

The curve is interesting in that two equivalence points are observed. These occur after addition of four and six moles of cyanide per mole of nickel(II) respectively, and demonstrate the formation of stable species $Ni(CN)_4^{2-}$ and $Ni(CN)_6^{4-}$. Since present indications from other experimental methods are that $[Ni(CN)_6]^{4-}$ (in aqueous solution) is not paramagnetic, this particular result raises other problems, because only paramagnetic solutes are thought to be effective in catalysing spin-lattice relaxation.

A feature which makes the technique attractive in analytical chemistry is the small amount of solute required. In our apparatus sample volumes of 0·23 c.c. and concentrations of nickel(II) in the range 0·01–0·1 M were convenient, but both these values could be substantially reduced. The dilution during titration can be kept low by using a concentrated solution of titrating agent, but is easily allowed for by multiplying the values of $(T_1^{-1} - T_0^{-1})$ by V/V_0, where V_0 is the initial volume of the solution and V the volume after addition of titrating agent.

Other applications of the titration technique, for example, to the study of relative stability constants of different complexes in solution, readily suggest themselves.

It is hoped to publish more detailed accounts of the technique and experimental results elsewhere.

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A Dose Test for Irradiated Polyethylene

ALTHOUGH a large body of results has been published on the changes of physical properties induced in ethylene polymers by irradiation, no description of an actual dose test has come to our notice. It happens during electron irradiation of objects that such factors as thickness may influence the dose received at any point so that it is desirable to have available a test which can be used to assess

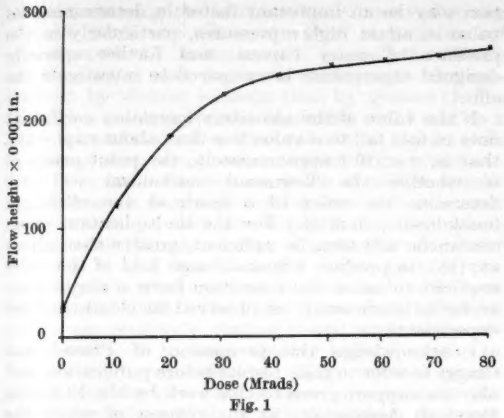

Fig. 1

the radiation dose received by a small amount of material.

In the case of polyethylene, Charlesby and Hancock[1] studied the effect of radiation dose on the relationship between elastic modulus and temperature. From this work a dose-evaluation test could easily be developed, but rather large test samples are required. Other work, which was the starting point for the present study, is due to Ballentine *et al.*[2] and to Lanza[3], who have shown that polyethylene acquires a progressive resistance to deformation with increasing radiation dose, and that the effect shows better at higher test temperatures. Their work does not go far enough for an accurate dose test to be derived from their results.

We have studied the deformation of irradiated polyethylene test pieces using a Williams plastimeter. Under a loading of 5 kgm. on a standard test piece at 150° C. the deformation in 3 min. varies with dose received up to 80 Mrad (Fig. 1). These particular data refer to 'Alkathene 2' (Imperial Chemical Industries, Ltd.) but similar results are obtained with other polyethylenes. The test can only be applied to samples from which cylindrical test pieces may be machined, or to sheets from which disks may be punched to make such a test piece; moulded test

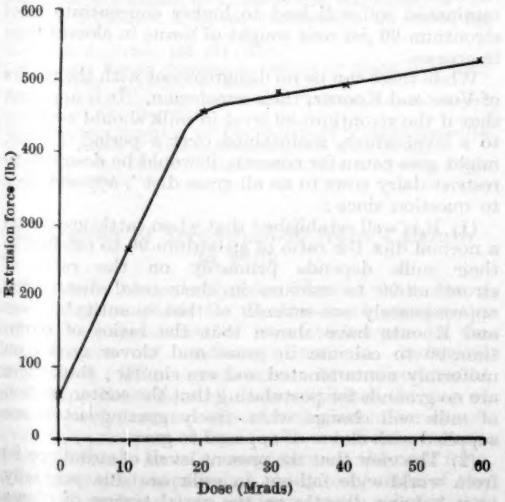

Fig. 2

pieces at 150° C. revert to their original shape before moulding, so giving irregular cylinders. This effect is due to the well-known 'elastic memory'.

To overcome this limitation another test has been developed in which the polyethylene is extruded at 150° C. using a Hounsfield tensometer with extrusion attachment. After a few moments the extrusion pressure settles down to a steady value which depends upon the dose received by the polyethylene (Fig. 2). These results refer to 'Alkathene 2' and also to highly expanded cellular polyethylene, which cannot be tested by the Williams plastimeter method.

The methods given appear to be suitable for testing all types of polyethylene for dose received where weights of only a few grams are available.

We are grateful to Mr. W. E. Simpson for helpful discussion and to Mr. T. R. Scott, director of research, for permission to publish this communication.

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March 16.

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Secondary Ionization in Humid Air

Recent measurements of Townsend ionization coefficients by Prasad and Craggs[1] in these laboratories have revealed large variations in the values obtained for the secondary ionization coefficient γ in discharge gaps in air containing water vapour. For example, in humid air at a total pressure of 150 mm. mercury with a partial pressure of 15 mm. mercury of water vapour, its value for a 'Dural' cathode is $7{\cdot}5 \times 10^{-3}$ at $E/p = 45$ V./cm./mm. mercury as compared with a value of $2{\cdot}3 \times 10^{-5}$ in dry air at the same total pressure and electric field.

On the assumption that the secondary ionization mechanism is attributable to photoelectric action at the cathode, as is generally considered to be the case in air at low values of E/p, then it is possible that the observed large reduction in the secondary ionization coefficient may be caused by the absorption of photons by the water vapour present. Its value may then be expected to vary approximately according to the relation:

$$\gamma = \gamma_0 \exp(-\mu p_w d)$$

where γ_0 is the value of γ at $p_w = 0$, p_w is the partial pressure of water vapour in millimetres of mercury, μ is the absorption coefficient at $p_w = 1$ mm. mercury and d is the gap-length in cm.

In order to check whether this relation holds in the case of humid air, the values of $\ln\gamma$ have been plotted as a function of $p_w d$, as shown in Fig. 1. The circled points denote the values of $\ln\gamma$ at different partial pressures of water vapour, up to 15 mm. mercury, in air at a total pressure of 150 mm. mercury. The crossed points denote the values at several gap-lengths, ranging from 1·3 to 4·2 cm., in air at a total pressure of 150 mm. mercury containing 9 mm. mercury pressure of water vapour. Both sets of points are roughly collinear, as is to be expected if photoabsorption is affecting the secondary emission from the cathode. The approximate value of the absorption coefficient, as determined by the slope of the line through the points, is $\mu \sim 0{\cdot}2$ cm.$^{-1}$ at $p_w = 1$ mm. mercury.

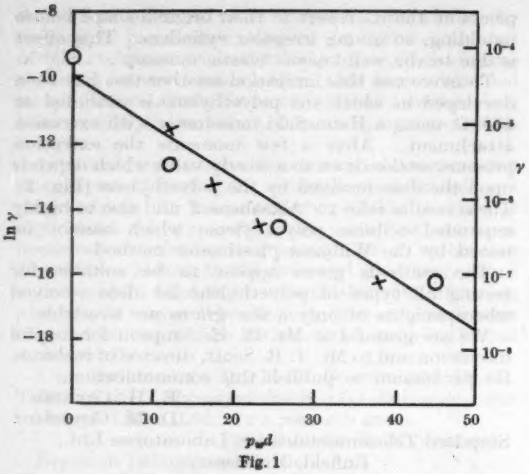

Fig. 1

Usually variations of the secondary ionization coefficient in Townsend discharge studies have been explained by consideration of changes in cathode work function, possibly caused by oxide layers or by adsorption of oxygen and other gases on the cathode surface. There is no doubt that surface conditions greatly affect it, and it is possible that in humid air a layer of water on the cathode surface could be responsible for the large fall in secondary emission. But the observation that the coefficient varies roughly exponentially with $p_w d$ suggests strongly that photoabsorption may also be an important factor in determining its value in humid air. This would not be inconsistent with other evidence as, for example, in Geiger counters, where absorption in methane has been found to reduce secondary photoelectric emission from cathodes. According to Weissler[2], absorption in methane does not occur at wave-lengths greater than about 1400 A., whereas in water vapour absorption is observed at wave-lengths up to about 1900 A. Raether[3] has also reported greatly reduced values of the secondary ionization coefficient in gases containing methane or alcohol.

Even in the absence of water vapour, it is possible that photoabsorption may affect the values of the coefficient of secondary ionization. The results for dry air show a steady fall in γ with decreasing value of the product of gas pressure p and the breakdown gap-length d. When the values of $\ln\gamma$ are plotted as a function of pd, over the range 100–600 mm. mercury × cm., a roughly linear relation is obtained with a slope giving $\mu \sim 8 \times 10^{-3}$ cm.$^{-1}$ at $p = 1$ mm. mercury. The result is complicated by the fact that the magnitude of E/p at breakdown increases with decreasing values of pd. Nevertheless, the suggestion that absorption may affect the coefficient of secondary ionization even in dry air does not appear unreasonable if one notes that, according to Weissler[2], absorption occurs in oxygen at wave-lengths up to about 1700 A.

The above preliminary views are put forward tentatively as a possible explanation of the variations observed by Prasad and Craggs in their measurements of the secondary ionization coefficient. As their experiments were not designed to examine directly the effect of absorption on the secondary ionization coefficient, the deductions that can be made based on their results are limited. There does appear to be sufficient evidence, however, to indicate that absorption may be an important factor in determining its value in air at higher pressures, particularly in the presence of water vapour, and further specially designed experiments are required to investigate the effect.

If the value of the secondary ionization coefficient does in fact fall to a value less than about $\exp(-18)$, that is, $\gamma < 10^{-8}$ approximately, the point arises as to whether the Townsend mechanism will still determine the onset of a spark at the minimum breakdown potential. For the multiplication in an avalanche will then be sufficient, greater than about $\exp(18)$, to produce a space-charge field of the order required to cause the transition from a single avalanche to a streamer, as observed in cloud-chamber experiments.

I acknowledge the permission of Prasad and Craggs to refer to their results before publication, and also the support given to this work by the Electrical Research Association, at the request of which the studies of ionization in humid air have been made.

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RADIOBIOLOGY

Uptake of Strontium by Pasture Plants and its Possible Significance in Relation to the Fall-out of Strontium-90

Vose and Koontz[1] have lately compared the absorption of strontium-90 and calcium by six species of clover and six species of grass grown in pots containing three contrasting soil types. Their results support the conclusion, previously advanced on less complete results[2], that plant species differ little in the manner in which they distinguish between strontium-90 and calcium. It is well known that the calcium content of clovers is usually higher than that of grasses. Thus, as Vose and Koontz have noted, the absorption of strontium-90 from uniformly contaminated soil will lead to higher concentrations of strontium-90 per unit weight of tissue in clovers than in grasses.

While there can be no disagreement with the results of Vose and Koontz, their conclusion, "It is apparent that if the strontium-90 level in milk should ever rise to a level which, maintained over a period of time, might give cause for concern, it would be desirable to restrict dairy cows to an all-grass diet", appears open to question since :

(1) It is well established that when cattle are fed on a normal diet the ratio of strontium-90 to calcium in their milk depends primarily on the ratio of strontium-90 to calcium in their total diet, being approximately one-seventh of that quantity[3]. Vose and Koontz have shown that the ratios of strontium-90 to calcium in grass and clover grown on uniformly contaminated soil are similar; thus there are no grounds for postulating that the contamination of milk will change when freely grazing cattle are supplied with clover as opposed to grass.

(2) The view that the present levels of strontium-90 from world-wide fall-out in milk are due primarily to it lodging directly on the aerial tissues of plants

and not entering the soil[2] has lately received strong support from the observation of high ratios of strontium-89 to strontium-90 in milk[4,5]. There is no evidence that the aerial entrapment of strontium-90 by clovers exceeds that by grasses; thus the higher calcium content of clovers could lead to a lower ratio of strontium-90 to calcium in clovers than in grasses when aerial contamination is important.

(3) In so far as the contamination of pasture species with fall-out is due to absorption from soil, it is most important to consider the depth from which plant roots absorb their nutrients; a particular reason for so doing is the fact that the major part of the strontium-90 which enters undisturbed soil from fall-out is retained in the upper 5 cm. of soil[6]. Pasture grasses are relatively shallow-rooted while the root-systems of some legumes, notably lucerne, extend to considerably greater depths. Experimental results accord with the view that in these circumstances the ratio of strontium-90 to calcium is least in the more deeply rooted species[7]. For this reason experiments in pot culture, such as Vose and Koontz have described, do not necessarily indicate the situation which will occur under field conditions.

Thus, whereas when plants absorb strontium-90 from uniformly contaminated soil the balance between grass and clover in pastures appears unlikely to be an important factor in determining the strontium-90 content of milk, the presence of clovers in pasture may, under other circumstances, reduce, rather than enhance, the strontium-90 content of milk.

No final statement can yet be made on many aspects of this subject, which is under intensive examination in a number of laboratories; at present, however, there appear to be no valid grounds for suspecting that the presence of clovers in contaminated pastures is likely to increase the radiation dose to the population.

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Separation of Strontium-90 from Calcium in Milk

Attention has recently been directed to the use of ion-exchange methods for alkaline earth separations and to the application of these techniques in the determination of radiostrontium in biological materials[1,4]. The measurement of environmental levels of radionuclides often necessitates the use of comparatively large samples of biological material and involves, in the case of liquid milk, a tedious evaporation procedure[2]. In an attempt to meet the need for a more convenient approach, a study was made of the extent of exchange, on cationic resin columns, of calcium and strontium absorbed from milk passed directly on to the columns.

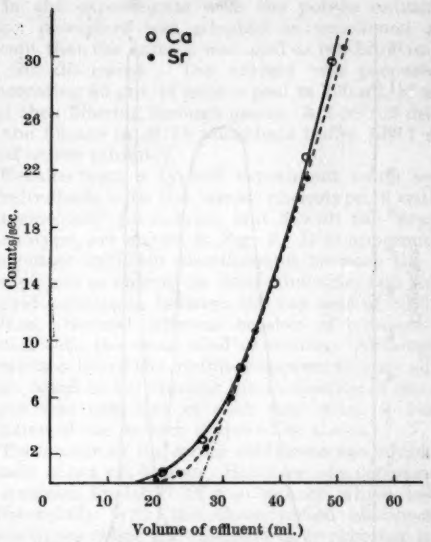

Fig. 1. Calcium, ○, and strontium, ●, breakthrough. Flow-rate, 0·14 ml./cm.²/min.; temperature, 22° C.; column-length, 7·5 cm.

Whole milk samples, in which the natural calcium and strontium were 'spiked' with calcium-45 (0·25 MeV.β) and strontium-85 (0·51 MeV.γ), were run on to columns of a cationic exchange resin ('Zeo Karb' 225, 50–100 mesh, sodium form) and measurements were made of the β- and γ-activities in known volumes of the effluent. The breakthrough capacity for calcium, as indicated by a significant rise in counting rate (Fig. 1), corresponded to the value expected on the basis of capacity determinations carried out by potentiometric titration with sodium hydroxide. Since the ratio of calcium to total strontium (that is, natural strontium and strontium-85) in the milk is high, the resin capacity will be measured primarily by the retention of the calcium component. The observed equality of capacity for the two ions indicates removal of strontium by the large excess of calcium. For volumes of milk containing insufficient calcium to produce resin saturation, gravimetric analysis on influent and effluent solutions showed a quantitative retention of calcium (experimental error ±1 per cent). From parallel estimations of the casein content[3] it was concluded that there was zero retention of casein (experimental error ±2 per cent) indicative of complete breakdown of the calcium–casein complex.

Investigation of the elution characteristics for absorbed calcium and strontium was made using ethylenediamine tetraacetic acid as elutriant. Variations in the eluate of the specific activity of calcium-45 and strontium-85 added to the milk were observed in small-volume fractions collected at defined intervals, and also by continuous monitoring of the eluate passing through a flow counter (20th Century Electronics). The results, illustrated in Fig. 2, for elution at *p*H 8 showed good recovery (>90 per cent) of both calcium and strontium fractions. Further tests, using a range of different *p*H values for the elutriant, gave results in keeping with those described by Davis[1]. No interference with

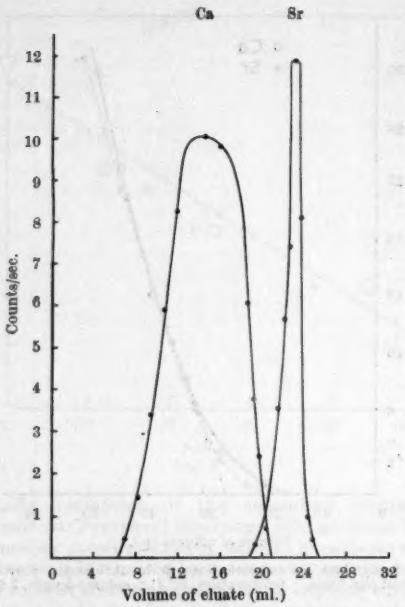

Fig. 2. Separation of calcium and strontium from 1 ml. of milk. Eluting solution: 1 per cent tetra sodium salt of ethylene diamine tetraacetic acid. *p*H 8. Flow-rate, 0·17 ml./cm.²/min.; column-length, 16·5 cm.; temperature, 22° C.

column operation was experienced from the milk-fat component, and the resin was readily regenerated for further use. These techniques may therefore be applied satisfactorily to the separation of calcium-free strontium in high yield directly from liquid milk.

I am indebted to Prof. W. V. Mayneord in whose Department the above work was carried out, and to the Medical Research Council for financial aid.

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BIOCHEMISTRY

Position of the Fatty Acids in Cardiolipin

Evidence was obtained previously[1-3] that cardiolipin is composed of two phosphoric acid, three glycerol and four fatty acid residues. According to this structure the polyglycerophosphate skeleton (1) has five hydroxyl groups available for esterification.

$$CH_2OH.CHOH.CH_2O.P : O(OH).CH_2O.CHOH.$$
$$CH_2O.P : O(OH).CH_2O.CHOH.CH_2OH \quad (1)$$

The position of the fatty acids has now been ascertained by identification of the fragments obtained on hydrolysis of cardiolipin in conditions in which splitting of the phosphoric diester bonds take place preferentially with little rupture of the fatty acid ester bonds.

Cardiolipin (620 μmoles phosphorus), prepared from ox heart muscle by the method of Faure and Morelec-Coulon[4], was heated 90 min. in 50 ml. 90 per cent (v/v) acetic acid at 100° C., and after removal of the acetic acid *in vacuo* the water-soluble and ether-soluble fractions were separated. The ether-soluble fraction contained 68 per cent of the total glycerol, 19 per cent of the phosphorus and 98 per cent of the total fatty acids, only 5 per cent of which were free. By chromatography on silicic acid according to Borgström[5] 76 per cent of the fatty acids were recovered in the diglyceride fraction (485 μmoles; molar ratio fatty acid–fatty acid ester–glycerol–phosphorus, 1·92 : 1·96 : 1·0 : 0; 1·7 double bonds/mole fatty acid); by periodic acid oxidation, not more than 2 per cent of this fraction was present as monoglyceride. The remaining fatty acids and the ether-soluble phosphorus were recovered in phosphatide fractions in partly degraded forms (phosphorus–glycerol–fatty acid, 1 : 1·1 : 2·2).

The water-soluble fraction of the hydrolysate contained 80 per cent (500 μmoles) of the phosphorus and 32 per cent (295 μmoles) of the glycerol, combined in a form hydrolysable by phosphomonoesterase; not more than 10 per cent of the phosphorus, however, was present as glycerolmonophosphate, as estimated by Burmaster's method[6], and no free glycerol was detected. On fractionation 80 per cent of the water-soluble phosphorus was recovered as a barium salt sparingly soluble in water, corresponding in composition to barium glyceroldiphosphate (phosphorus–glycerol–barium, 2·0 : 1·06 : 2·2).

The main products of hydrolysis were therefore glyceroldiphosphate and diglycerides in molar proportion 1 : 2. These results are incompatible with the structure proposed for cardiolipin by Pangborn[7] (a chain of four glycerol and three phosphoric acid residues, in which all six available hydroxyl groups are acylated), which entails the formation on hydrolysis of free fatty acid in proportion to the water-soluble phosphoric ester formed. The results are consistent with a structure for cardiolipin as a derivative of 1, in which the terminal glycerol residues are esterified as diglycerides, leaving a free hydroxyl group on the middle glycerol residue.

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Differential Response of Human Serum Cholinesterase Types to an Inhibitor in Potato

Differences in the degree of inhibition of the activity of human serum cholinesterase (pseudocholinesterase) produced *in vitro* by the local anæsthetic dibucaine (nupercaine) make it possible to classify individuals into three distinct types according to the character of the cholinesterase present in their serum[1]. These types are inherited, and are thought to be determined by a pair of allelic genes each of which is responsible for the synthesis of a distinct form of

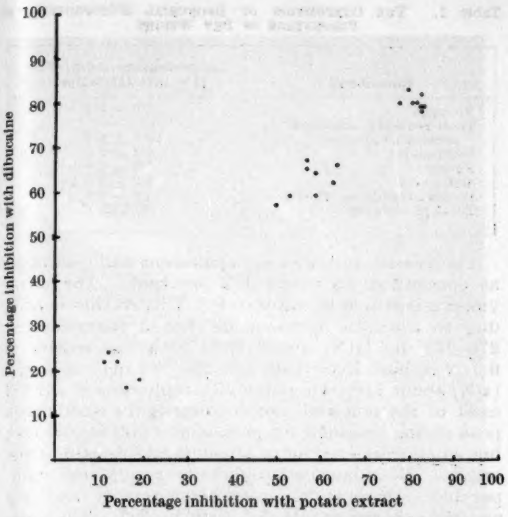

Fig. 1. Relation between percentage inhibition with dibucaine and with a potato-peel extract of pseudocholinesterase in plasmas from 21 different individuals

serum cholinesterase[2]. The two types of homozygotes each have only one of these esterases in their serum, while the heterozygote is believed to have both of them in about equal amounts[2]. The classification of the three phenotypes may be achieved by a number of cholinesterase inhibitors besides dibucaine[3]. However, there also exist certain cholinesterase inhibitors, such as tetraethylpyrophosphonate and di*iso*propylfluorophosphonate which fail to discriminate between the three types, presumably because their mode of action is different[3].

Orgell, Vaidya, and Dahm[4] have recently demonstrated that aqueous extracts of a number of solanaceous plants are capable of inhibiting human serum cholinesterase. It was of interest to see whether or not this common naturally occurring inhibitor acted differently on the three genetically determined phenotypes. A convenient source of this inhibitor is potato, and in the present experiments extracts of potato peel were used because it had been shown that the concentration of the inhibitor was considerably greater in the peel than in the inner part of the tuber.

A series of plasmas from individuals of the three cholinesterase phenotypes were used as a source of the enzymes. They had been typed according to the method of Kalow and Genest[1], using a 1-in-100 dilution of plasma, with 5×10^{-5} *M* benzoylcholine as substrate, 10^{-5} *M* dibucaine as inhibitor, in *M*/15 phosphate buffer *p*H 7·4. The reactions were followed spectrophotometrically at 240 mμ. The percentage inhibition obtained under these conditions was 77–83 per cent for the so-called 'usual' phenotype, 53–67 for the 'intermediate' phenotype, and 16–24 for the 'atypical' phenotype. The plasmas of the uncommon 'atypical' phenotype were mainly obtained from individuals who had been found to be unusually sensitive to the muscle relaxant succinylcholine when this was administered during anæsthesia. The individuals of the 'intermediate' phenotype were identified either in the course of studies of the families of the 'atypical' individuals or during random population surveys, where they constitute about 3 or 4 per cent of all people tested.

In the experiments with the potato extract the same procedure was adopted as mentioned above except that the extract was used as inhibitor in place of the dibucaine. The extract was prepared by macerating 85 gm. of potato peel in 300 ml. of water, and then filtering through gauze. A 1-in-100 dilution of the filtrate in *M*/15 phosphate buffer *p*H 7·4 was used as the inhibitor.

Results from a typical experiment using sera of 8 individuals with the 'usual' phenotype, 8 with the 'intermediate' phenotype, and 5 with the 'atypical' phenotype, are shown in Fig. 1. It is apparent that the potato inhibitor discriminates between the three groups just as sharply as does dibucaine, and there is a good correlation between the two sets of inhibition values. Several different batches of potatoes were tested with the same kind of results. Although the concentration of the inhibitor appears to vary slightly from batch to batch, clear discrimination of the three types was obtained in each case using a 1-in-100 dilution of the extract prepared as above.

The nature of the serum cholinesterase inhibitor in potato is not yet known. However, the occurrence in a common foodstuff of a substance which behaves differentially with the three serum cholinesterase phenotypes raises the question as to whether it may not be connected in some way with the genetical polymorphism determining the occurrence of alternative forms of serum cholinesterase in human populations.

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An Alteration in the Excretion of Free Serine in Urine from Irradiated Humans

A number of investigators have been interested in the urinary excretion of amino-acids following total-body irradiation[1]. No one has used the quantitative column procedure of Moore, Spackman and Stein[2]. This preliminary communication is based on their procedure.

In June 1958 eight workmen were accidentally exposed to total-body gamma and neutron irradiation at one of the U.S. Atomic Energy Commission's installations in Oak Ridge. These men were studied at the Medical Division of the Oak Ridge Institute of Nuclear Studies. During the first 16 days the clinical manifestations of irradiation were minor, and the patients were ambulatory and ate well. There was a slight gain in weight during this period.

Urine was refrigerated (about 5° C.) after it was voided and, when a 24-hr. collection was complete, the volume was measured and aliquots for various procedures taken. As soon as the measurement and sampling was complete, all specimens were stored in the deep-freeze about (−15° C.) until they were analysed. Aliquots (2 ml.) of filtered urine were analysed without desalting. A commercial model of the automatic apparatus of Spackman, Stein and Moore[2] was used. Other analyses and analyses of hydrolysed urine[4] were done with the fraction-collector method[2].

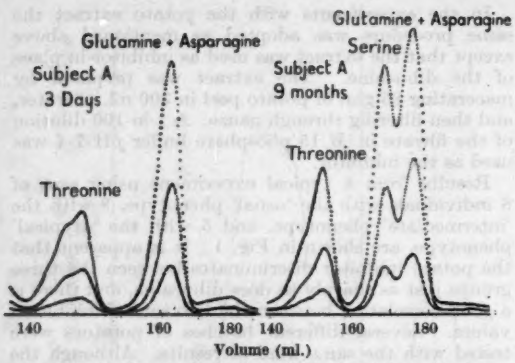

Fig. 1. Part of a recorder tracing of ninhydrin amino-acid colour taken from analyses of urinary amino-acids excreted by a man who was accidentally exposed to 365 rad of whole-body gamma and neutron irradiation. Serine is absent from the urine excreted 3 days after exposure

Among the most interesting results is the decrease in urinary excretion of free serine. This decrease is illustrated in Fig. 1. An analysis of a sample of urine excreted nearly nine months after the accident that shows the presence of free serine is included for comparison. Free serine was decreased even on the first day after irradiation and was apparently absent from this patient's urine three days after exposure. Traces only were excreted on the second and tenth days after irradiation. On the fourth and sixteenth days no serine was excreted. Serine was present in the urine after acid hydrolysis.

Serine was decreased but not absent from urine excreted by a child with leukæmia during the second day after 800 r. total-body irradiation. This irradiation was from a cobalt-60 source and, therefore, pure gamma. In contrast, there was a considerable neutron component in the accidental irradiation.

We do not have analyses of plasma and, therefore, do not know whether the reduced serine excretion is a renal effect or whether there is a change in the intermediary metabolism of this amino-acid in irradiated humans.

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Mucoprotein of Bronchial Mucus Gel

In the absence of acute infection, the excessive bronchial secretion in chronic bronchitis is mucoid and owes its viscidity to a gel structure. This is apparent as an oriented fibrous network in the polarizing and electron microscopes[1–3], and there is a yield-value for disruption of the gel[2–4]. Mucoprotein can be isolated in yields of about 60 per cent of the total nitrogen by the procedure of Curtain and Pye[5], and has the composition shown in Table 1. Mannose and galactose are also constant constituents[1–3].

Table 1. The Composition of Bronchial Mucoprotein, as Percentage of Dry Weight

Constituent	Gm. percentage of dry-weight M ± *S.D.* (13 samples)
Nitrogen	8·6 ± 1·1
Total reducing sugar, expressed as glucose	19·5 ± 4·9
Hexosamine	6·1 ± 1·7
Fucose	5·1 ± 1·3
Sialic acid	4·2 ± 1·3
Bound sulphate as sulphur	0·0 – 0·2
Total phosphorus	*c.* 0·25

The viscous solutions are opalescent and gelatinous at concentrations above 1–2 per cent. The ultraviolet absorption in water or 0·1 *N* hydrochloric acid, due to aromatic amino-acids, has a maximum at 270–275 mμ (ε(N) about 190), with less scatter in 0·1 *N* sodium hydroxide and 280–284 mμ maximum (ε(N) about 110). On paper electrophoresis at *p*H 8·6 most of the material moves towards the anode as a peak giving reactions for protein and polysaccharide; but small fractions run in advance and remain at the origin. Boundary-electrophoretic results are comparable; there is no gross heterogeneity and less negative charge at *p*H 4·5 than at 8·5. The composition and properties are similar to those found by Brogan[6] for mucoprotein in the insoluble fraction after acid-peptic digestion of acetone-dried sputum.

In Ostwald viscometers there is an exponential relationship of relative viscosity to concentration up to 1 per cent (w/v). Although this is similar to deoxyribonucleic acid, there is no evidence for more than traces of this substance in the mucoprotein, and deoxyribonuclease does not affect the viscosity in the presence of magnesium ions. The intrinsic (limiting) viscosity of the mucoprotein has been determined for dilute, salt-free solutions (0·001–0·025 gm. protein/100 ml.), using Ostwald viscometers B.S. 1 and 2 at 25° C., and applying a kinetic energy correction to No. 2. Non-Newtonian at higher concentrations, solutions of mucoprotein below 0·02 gm. protein/100 ml. give comparable viscosities at different rates of shear. Plotting the reduced specific viscosity (η_{sp}/c) against concentration (c) and extrapolating fitted lines to zero concentration, the values for intrinsic viscosity are 8·1 (± 0·07) from viscometer 1, 8·8 (± 0·57) from viscometer 2, and do not differ within the limits of the sampling errors[7]. The partial specific volume is 0·69 by pycnometry. This gives an average viscosity increment of 1225, corresponding to an axial ratio[8] of about 150. Although this has little precision, since hydration is unknown, orientation of flow is likely to have occurred, and the electron-microscope suggests flexible chains and a greatly elongated molecule is indicated. Similar results were obtained by Curtain[9] for urinary mucoprotein.

Physical differences exist between bronchial and urinary mucoproteins. Curtain[9] found that solutions of the latter increase in viscosity with increasing ionic strength; precipitation is maximal in 0·58 *M* sodium chloride[6,10]. Very low concentrations of ions lower the viscosity of bronchial mucoprotein; with sodium chloride, viscosity is minimal at 0·04 *M*, increasing very slightly up to 4 *M*, without precipitation. The behaviour on centrifugation suggests modification of a gel structure. The aqueous 'solutions' are stable on standing at 5° C. (though occasionally an insoluble syneresed coagulum separates), but a loose gelatinous deposit forms with quite low centrifugal force (about 60 per cent of the protein

Fig. 1. Electron-micrograph of bronchial mucoprotein, shadowed with gold–palladium. (× 22,500)

at 9,000 r.p.m. for 1 hr.); in 0·15 *M* sodium chloride, more than 75 per cent of protein deposits in compact form. These deposits redisperse readily. Agents which modify hydrogen-bonding cause a fall in viscosity comparable to dilute salts, but very little deposits on centrifugation; for example, 6 *M* urea, 3·2 *M* guanidine hydrochloride, and particularly 8 *M* lithium bromide. In the ultra-centrifuge at speeds between 4 and 50 × 10^3 r.p.m., and at protein concentrations of 0·2 and 0·02 gm./100 ml. of 0·04 *M* sodium chloride, the concentration of the bulk of the solution diminishes rapidly, but with little movement of the peak. Gel deformation in the centrifugal field may well account for this. Tamm, Bugher and Horsfall[11] obtained $S_{20} = 29·5$ for urinary mucoprotein and molecular weight $7 = 10^6$; however, there is likely to be a range of molecular size in this material also[10].

The bronchial mucoprotein forms oriented inclusions in crystals of sodium chloride[1,12]. By electron microscopy, the fibrils range in diameter from 0·1–0·2μ to 400 A. Between these, smaller, flexible microfibrils are seen (Fig. 1), and measurement of gold–palladium-shadowed preparations indicates that these are ribbon-like with mean cross-sectional dimensions, 304 A. wide by 74 A. thick, but occasionally about half this size. Although certainly composed of several molecular units, these approach the fundamental dimensions for a number of stable fibriform bodies[13]. A beaded appearance in the smaller micro-fibrils possibly indicates sub-units. There are similarities between the micro-fibrils and those of the urinary mucoprotein of Porter and Tamm[14].

In conclusion, it seems that the gel-structure of bronchial mucus is still present in the mucoprotein 'solutions' derived from it.

Note added in proof: Recent electron-microscopic evidence indicates breakdown of the gel-structure into short, rigid sub-units in 8 *M* lithium bromide.

We are grateful to Drs. R. Markham and E. M. Shooter, and to Prof. R. D. Preston for facilities for ultra-centrifugal, electrophoretic and electron-microscopic studies, and to the Peel Trust for a grant.

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Phosvitin in the Serum of the Hen

A PHOSPHOPROTEIN fraction appears in the serum of the hen in association with the onset of laying[1,2] or in the serum of the immature bird on treatment with œstrogen[3]. It can be shown by zone electrophoresis on filter paper that two new major protein fractions appear in the serum on œstrogenization, one of these being specially rich in protein phosphorus[4]. The demonstration by Mecham and Olcott[5] of the existence of phosvitin in egg-yolk, and the view that yolk constituents are synthesized in the liver and transferred thence to the ovary in the blood plasma, prompted us to examine the blood of the hen for presence of phosvitin.

Four laying hens were each given 2 mgm. œstradiol benzoate per day for 5 days. The birds were then bled and 200 ml. serum was recovered. Crude lipovitellin complex was precipitated by bringing the serum to *p*H 5·3 and then adding an equal volume of distilled water. The precipitate was separated on the centrifuge, re-dissolved in 1 per cent sodium chloride and re-precipitated by ten-fold dilution with distilled water. The precipitate was worked up for phosvitin by the method of Joubert and Cook[6] as though it were egg-yolk, with the exception that an international refrigerated centrifuge Model *PR*1 was used instead of a high-speed centrifuge as used by Joubert and Cook. The method of Joubert and Cook avoids complications introduced by ether extraction.

After the final dialysis, the water-clear supernatant yielded, on freeze-drying, 150 mgm. of a white protein that contained 10·1 per cent phosphorus and 12·3 per cent nitrogen. The protein behaved as a single component and displayed the same mobility on paper electropherograms as did samples of phosvitin prepared from egg-yolk by the same technique, and gave no lipid stain.

We believe that this result represents the first occasion on which phosvitin has been identified as a constituent of the hen's blood. We consider further that the slow-moving 'PP' lipoprotein fraction, rich

in protein phosphorus, on electropherograms of sera of œstrogenized and laying birds[4] probably represents a lipovitellin–phosvitin–γ-livetin complex in the sense in which this term is used by Joubert and Cook[6]. The 'PP' fraction was originally distinguished by the use of methanolic veronal buffer, but more recently we have been able to distinguish it by zone electrophoresis in aqueous buffer (*p*H 8·6, μ 0·05). The second, somewhat more mobile, lipoprotein fraction ("fraction 8" of Vanstone *et al.*[7]) presumably corresponds mainly to lipovitellenin.

We wish to thank the Agricultural Research Council of Quebec for its support of this project through a grant-in-aid of research. It is hoped to publish a fuller account at a later date.

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Basic Substances as Synergists for Fat Antioxidants

Added fatty acids decrease the effectiveness of a number of antioxidants in marine and other oils and fats[1]. It has now been demonstrated that many oil-soluble basic substances may act as synergists. For example, a primary amine (octadecylamine, ODA), a secondary amine (proline), and a tertiary amine (tri*iso*octylamine, TOA) provided unexpectedly great protection against oxidation to oils containing added 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (EMQ, 'Santoquin'), whereas in the absence of the last-named they conferred only slight or no protection at the same levels (Table 1).

The effects of the various basic additives differed with each antioxidant. For example, in general they were synergists to 2-tertiarybutyl-4-methoxyphenol (BHA) but had no effect or acted as antagonists to 2,6-ditertiarybutyl-4-methoxyphenol (BHT). They were also more effective with γ-tocopherol than with α-tocopherol. In this respect they have been found useful in differentiating the various tocopherols with regard to antioxidant effectiveness.

Substrates also modified the respective activities. With the samples of highly purified trilinolein available to us (Hormel Foundation), octadecylamine acted as an antagonist to α-tocopherol and synergist to γ-tocopherol, whereas in menhaden oil octadecylamine was synergistic with both.

Proline was particularly effective in vegetable oils, probably acting in conjunction with the natural antioxidants. For example, samples of a refined olive oil containing 0, 0·025 and 0·05 per cent added proline had induction periods at 60° C. of 4, 12 and 23 days, respectively.

The synergistic effects of phospholipids and of some amino-acids on phenolic type antioxidants have been recognized for a number of years[2]. It appears likely that these effects may be mediated through the basic groups of such compounds, modified by solubility, heavy-metal chelating, and other properties. With respect to phospholipids, this contrasts with a previous suggestion[3] that the phosphate portion of the molecule was primarily involved.

Table 1. Effect of Mixtures of Antioxidants with Basic Synergists on Menhaden Oil and Trilinolein

Antioxidant	Basic additive	Induction period*–Days (50° C.)	
		Menhaden oil†	Trilinolein
EMQ	None	3	31
,,	ODA	28	56
,,	Proline	87	61
,,	TOA	104	41
,,	Phospholipid‡	33	—
BHA§	None	2	12
,,	TOA	11	15
BHT	None	4	19
,,	TOA	2	12
α-Tocopherol	None	2	10
,,	ODA	3	6
,,	Proline	3	18
,,	TOA	17	7
γ-Tocopherol	None	3	13
,,	ODA	9	31
,,	Proline	25	—
,,	TOA	36	36
None	None	1	1
,,	ODA	1	1
,,	Proline	1	1
,,	TOA	1	1

* Induction periods were determined by the gain in weight method recently described (ref. 4). 10-ml. beakers containing 200 mgm. substrate plus 0·5 μM of antioxidant and 2·5 μM of basic additive were incubated in a draft oven at 50° C., cooled and weighed once daily (to 0·1 mgm.). The end of the induction period was usually readily detected by a rapid gain in weight and arbitrarily fixed at the time at which the weight gain was 1 mgm.

† The menhaden oil was a commercial alkali-refined product treated to remove peroxides as follows: a 10 per cent solution in light petroleum ether was passed through a column of deactivated alumina and the solvent removed *in vacuo*.

‡ A preparation of yeast lecithin containing 1·89 per cent nitrogen, 4·1 per cent phosphorus, 0·03 per cent amino-nitrogen (D. J. Hanahan).

§ BHA is often designated 2 (or 3)-tertiarybutyl-4-methoxyphenol. The preparation which was used in much of this work contained at least 85 per cent of the 2-tertiarybutyl derivative. Similar results were obtained with the pure 2-isomer.

The progress of this work has been hampered by difficulties in obtaining reproducible runs. The results shown are, however, typical of a number of determinations. Details will be published elsewhere.

This work is supported in part by funds made available by the Saltonstall-Kennedy Act, administered by means of a collaborative agreement between the U.S. Fish and Wildlife Service and the University of California.

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An Improved Method for preparing Pectate Gels

The routine use of pectate gel media as an aid to the detection of bacteria causing soft rot of plant tissue has been restricted by the difficulty commonly encountered in their preparation. One of the most useful methods is that of Wieringa[1], who adopted the double-layer technique, consisting of a plate of calcium agar over which the pectate solution was poured. Diffusion of calcium ions from the agar brought about the formation of a gel.

In practice it has been found that convenient quantities of liquid pectate solution are difficult to prepare, as a weak gel forms on cooling and during storage. This gel frequently fails to liquefy uniformly on heating, and the resultant lumpy fluid produces unsatisfactory plates. Moreover repeated autoclaving of pectate solutions tends to lower the quality of the final gel.

The addition of disodium salt of ethylenediamine tetraacetic acid in low concentration to the pectate solution prevents the unwanted gel formation. This free-flowing liquid can be autoclaved normally and sterile stocks can be stored indefinitely without losing the ability to form a firm gel in the presence of sufficient calcium ions.

This appears to be due to the activity of the disodium salt as a chelating agent for the calcium ions present in the pectate. The high concentration of these ions diffusing from the calcium agar is sufficient to replace the calcium removed by chelation and, if in excess, to bring about adequate gelling of the pectate.

The procedure which has proved satisfactory in current work on soft rot pseudomonads can be summarized as follows. (*a*) Calcium agar base: peptone (Evans) 0·5, lemco 0·5, calcium lactate 0·5, agar (Davis) 1·5 per cent (sterilize at 15 lb. pressure). (*b*) Pectin solution: mix 2 gm. pectate powder with absolute alcohol to form a thin paste; make up with distilled water to 100 ml. and add 0·1 gm. disodium salt of ethylenediamine tetraacetic acid. Sterilize at 15 lb. pressure. The pectin solution is poured as a thin layer over a plate of the calcium agar (1 cm. deep). The gel sets firmly within 12–24 hr. at 27° C.

The pectate powder used was either 'Sodium polypectate' ('Exchange' brand obtainable from Sunkist Growers (Products, Ltd.), Ontario, California, and S. and S. Services. 72 Victoria Street, Westminster, London, S.W.1) or 'sodium pectate' (A.S.P. Chemical Co., Ltd., Bulstrade, Gerrard's Cross, Bucks).

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Conversion of Caffeic Acid to Esculetin during Paper Chromatography

Esculetin was found in an extract of plant tissue which contained caffeic acid. The esculetin was not a natural product but was formed from caffeic acid during the paper-chrcmatographic purification procedures. This conversion was confirmed by two-dimensional chromatography of authentic caffeic acid.

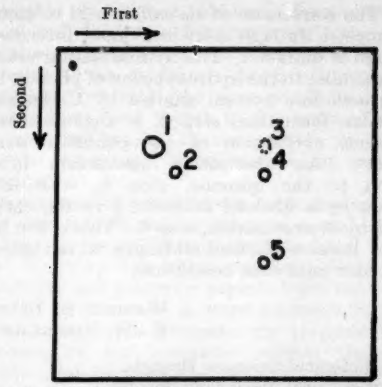
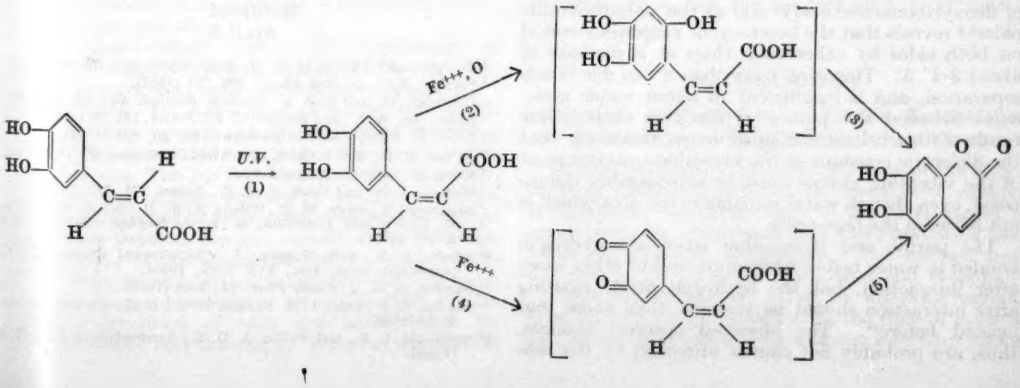


Fig. 1. Chromatogram of caffeic acid. Developing solvent: 5 per cent acetic acid in both dimensions

The fluorescent spots found on a two-dimensional chromatogram of caffeic acid using 5 per cent acetic acid as the solvent in both dimensions are shown in Fig. 1. When the chromatogram is developed in the dark on unwashed or water-washed chromatographic paper, only spot 1 is seen. If the caffeic acid is irradiated with a low intensity ultra-violet lamp for 5 min. at the origin before development, all five of the fluorescent spots are found. If chromatographic paper which has been washed with 0·001 *M* ethylenediamine tetraacetic acid is used, only three of the spots, 1, 3 and 5, are found on the chromatogram of the ultra-violet-irradiated caffeic acid. If the paper washed with ethylenediamine tetraacetic acid is dipped into 0·01 *M* ferric chloride and dried, the developed chromatogram of the ultra-violet-irradiated caffeic acid has all five spots. The spots were identified as: (1) caffeic acid; (2) esculetin; (3) caffeic acid; (4) esculetin; and (5) caffeic acid.

The ultra-violet light converted a part of the *trans*-caffeic acid at the origin to the *cis*-isomer[1]. Chromatography in the first dimension separated the slower moving *trans*-isomer from the faster moving *cis*-isomer. In the second dimension, a small amount of the *cis*-isomer reverted to the *trans*-isomer (spot 3 is quite faint) and esculetin appeared. The formation of the esculetin from the *cis*-caffeic acid is an oxidative reaction requiring metal ions. Esculetin is not found on chromatograms which are developed on ethylenediamine tetraacetic acid washed chromatographic



paper. The conversion of *cis*-caffeic acid to esculetin could proceed through a hydroxylated intermediate or through a quinone. The hydroxylation reaction, step 2, is similar to the hydroxylation of phenols by an ascorbic acid–iron system studied by Undenfriend[2]. The lactone formation, step 3, is analogous to the spontaneous cyclization of *cis*-*o*-coumarin acid to coumarin[1]. An alternative mechanism involves oxidation to the quinone, step 4, with lactone formation by a Michael addition β to the carbonyl followed by aromatization, step 5. This is less likely, however, because Michael additions do not generally occur under mild acid conditions.

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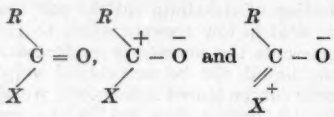
A Proposed Interpretation of Infra-red Spectral Changes occurring upon the Interaction of Polynucleotides

It has been found that the sodium salts of polyadenylic and polyuridylic acids interact[1–4] with formation of a two-stranded helix[1] with a structure very similar to deoxyribonucleic acid. More recently it was observed that the infra-red spectrum[5] of the mixture of these two acids showed the following marked changes from the summation of the curves of the components: a 39 per cent reduction in intensity of the 1,627 cm.$^{-1}$ band and a 7 cm.$^{-1}$ shift to higher frequency; a decrease in peak height (because of strong overlap the band was not integrated) and an 11 cm.$^{-1}$ shift to higher frequency of the 1,661 cm.$^{-1}$ band. The interaction of the sodium salts of polycytidylic and polyinosinic acids[6] has now been observed in the infra-red[7] with the following result: a marked decrease in intensity of the 1,651 cm.$^{-1}$ peak of the sodium salt of polycytidylic acid (the intensities have not yet been put on a quantitative basis) and a 19 cm.$^{-1}$ shift to higher frequency of the 1,678 cm.$^{-1}$ peak of the sodium salt of polyinosinic acid.

It is possible that an understanding of these changes may be reached by taking into account the change in environment of the vibrating groups after interaction has occurred. A consideration of the helical structure of deoxyribonucleic acid[8,9] and of the polynucleotide pairs[1,6] reveals that the heterocyclic rings are covered on both sides by other such rings at a distance of about 3·4° A. This is no more than a van der Waals separation, and is insufficient to admit water molecules between the planes of the two rings. One result of this helical configuration is, therefore, that the dielectric constant of the immediate environment of the vibrating groups must be considerably diminished, even though water remains in the area which is not between the base pairs.

The purine and pyrimidine bases are hydrogen bonded to water before interaction and to other bases after interaction, but the hydrogen bonds existing after interaction should be stronger than those that existed before[10]. The observed spectral changes, thus, are probably not caused (directly) by the new hydrogen bonds since the usual effect of hydrogen bonding is to decrease frequency and increase intensity rather than the reverse.

The following qualitative considerations, on the other hand, suggest that the observed changes are consistent with a decrease in dielectric constant. The principal resonance forms of the carbonyl group[11] are:



Hydrogen bonding favours the dipolar forms with a resulting shift to lower frequency. A marked decrease in dielectric constant of the medium, however, should diminish the contribution of the forms involving a charge separation and result in a shift to higher frequency. A second result of a decrease in dielectric constant is that there should be a smaller change in dipole moment with the vibration of the bond responsible for the absorption and a consequent reduction in intensity. Experimental results in support of these expectations are cited below.

In studies of frequency and intensity changes in approximately a dozen solvents[12,13] (see also ref. 11), it has been shown that the carbonyl stretching bands show a general trend of increase in frequency and decrease in intensity as the dielectric constant of the medium is decreased. Thus, for example, the frequencies of several ketones increase 11–14 cm.$^{-1}$ on going from chloroform to hexane and 8–9 cm.$^{-1}$ on going from benzene to hexane. There are also 18–36 per cent and 15–20 per cent reductions in intensity for the same solvent changes. *NN*-Diethylacetamide[12] showed a frequency increase of 38 cm.$^{-1}$ for the first solvent change and 15 cm.$^{-1}$ for the second, with intensity reductions of 24 and 20 per cent, respectively. *NN*-Diphenylacetamide showed a similar trend for those solvents in which it was soluble. The directions and approximate magnitudes of these spectral changes are thus consistent with those observed with the polynucleotides. While there are undoubtedly other factors contributing to the observed spectral changes, it is suggested that the above-mentioned decrease in dielectric constant may very well be the most important cause.

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OPHTHALMOLOGY

Regeneration of the Lens of the Eye in the Rabbit

In a recent communication, Chanturishvili[1] reports his re-investigation of the detailed embryology of the lens in Amphibia and refers to work on higher forms in his laboratory at Tiflis. He and his fellow-workers, in particular Sikharulidze[2], are of the opinion that the lens fibres are derived from cells which are neural in origin, and which require for the initiation of fibre production the products of the cytolysis of epidermal elements.

As a corollary of their view of lens development the Tiflis workers have introduced small fragments of cytolysing fœtal tissues into the lens capsules of adult animals from which all easily separable contents had been evacuated. They report that whereas simple evacuation without implant is followed by very little regeneration (the familiar aphakic state following the operation of extracapsular lens extraction), the addition of the implant is followed by the regeneration of a lens nearly normal in size and structure.

On a visit to Tiflis in 1955 one of us (P. G. 'E.) was enabled, by the courtesy of Prof. Chanturishvili, to see something of this work there. Later, Prof. Chanturishvili most kindly placed at our disposal, in the fullest possible detail, particulars of the techniques employed by him and his co-workers.

At Prof. Chanturishvili's suggestion, we repeated here the experimental implantation of cytolysing fœtal tissue taken from the lid commissure of a three-week embryo into the evacuated lens capsules of six normal rabbits according to the technique of Tamara Sikharulidze. We kept suitable control animals in which the lens capsule contents had been simply evacuated without implantation. This work was done because it was hoped that Prof. Chanturishvili would be able to attend the Oxford Ophthalmological Congress and to demonstrate this material of ours there. In the event, he was not able to do so, but these rabbits were demonstrated there by us in July 1958, 12 weeks after operation.

At that time there were marked differences to be seen between those evacuated lenses which had received implants and those which had not. Whereas the animals without implants showed the normal aphakic post-operative condition of collapsed and apposed capsular walls, those which had received implants showed inflated and in some cases spheroidal lens capsules, the contents and shape of which had a refractive power approximating to that of normal lens. This process of regeneration is continuing eleven months after operation.

Prof. Chanturishvili visited this Department in August 1958 and allowed us to examine his embryological preparations and generously presented us with material. He states that about 16 months are needed for full regeneration, but our most promising rabbits have lenses already little different from the normal in size. The iris can be seen to be supported by the convex anterior surface of the lens as in the normal eye, whereas in the aphakic controls the unsupported iris presents a concave surface to the observer.

Assessment of the structural and functional state of each lens in an ophthalmological sense has been made at frequent intervals by evaluation of the refractive condition of each eye as a whole by ophthalmoscopy and skiascopy. Whereas the normal unoperated eye in our rabbits is from zero to two and a half dioptres hypermetropic, the aphakic control eyes have consistently been found to be of the order of ten to fifteen dioptres hypermetropic. By notable contrast, the eyes which had received implants have been found, in the areas of incipient lens regeneration, to be comparable in refractive power to the normal as early as eight weeks after operation.

The progressive variations in shape and appearance of the lens capsule and its contents in each case have also been surveyed by frequent slit-lamp biomicroscopy. In every lens which received a fœtal implant the anterior and posterior capsule walls have become separated in part, and in some instances fully. The lens has assumed a progressively spheroidal shape. Relucence of the substance within the capsule suggests that it possesses optical properties comparable with those of a normal lens and differing from those of aqueous humour.

The approximately lenticular shape of the most successful examples and their relatively transparent and homogeneous contents make possible a satisfactory ophthalmoscopic view of fundus detail. Even in the best, however, there are some variations of optical density still remaining. These may possibly be ascribed to incomplete cytolysis of implanted material, or to incomplete removal of the original contents of the lens capsule. On the other hand, some of them may represent a stage in a process of regeneration not yet complete. Distortions of shape and incomplete separation of the capsule walls, which were found particularly in our first few operations, are probably due to faulty technique.

The preliminary observations reported here seem so far to confirm the findings of the Tiflis workers in the rabbit.

We propose to repeat our work here on a larger scale to allow the sectioning of regenerating lenses at all stages. It seems to us that this filling out of the capsule in so short a time may perhaps be ascribed to the secretion of a 'ground substance', in which fibres may grow later, more probably than to actual immediate growth of functional fibres.

Sikharulidze reports retarded but satisfactory regeneration of lenses in rabbits after the removal of cataracts induced chemically. At present we are engaged in a repetition of our work using rabbits which have received high doses of local irradiation.

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PHYSIOLOGY

Induction of Arginase in Rabbit Epithelium by the Shope Rabbit Papilloma Virus

In studies of the mechanism of action of the Shope virus in causing papillomas on the skin of rabbits, comparative tests were made of active transport of amino-acids into papilloma cells and into normal and hyperplastic rabbit epithelium. The method used was that recently reported by Rogers and Woodhall[1].

Papilloma epithelium taken at intervals varying from 10 days to one month from the time of inoculation of the papilloma virus was cut into tiny pieces,

the largest size of which was on the average of 0·25 mm. in diameter, rinsed in Ringer's solution, 50–100 mgm. amounts of tissue added to 1·5 ml. of a synthetic tissue culture media (Media 199, Difco), incubated for 2–20 hr. at 37° C., the cells and cell clumps centrifuged out of the media, the media chromatographed, and the relative amounts of amino-acids remaining determined semi-quantitatively with a Spinco analytrol densitometer. The chromatograms were made using butanol, 40; acetic acid, 10; water, 50 and aqueous phenol. Normal and hyperplastic skin were entirely similarly treated. The hyperplastic skin was of two sorts; the 'spontaneous' variety associated with cyclic hair growth and that induced by several treatments of the skin with a 50 : 50 solution of turpentine and acetone. Experiments were done in both Kansas cottontails and domestic rabbits.

A number of differences in the relative amounts of amino-acids removed from the media, as well as differences in which amino-acids were removed, were found. This communication is concerned with only a seemingly qualitative difference relating to arginine uptake. In repeated experiments the papilloma tissue invariably has taken up large amounts of arginine. No demonstrable uptake of arginine has been found in the case of normal or hyperplastic skin. However, as this amino-acid is a common constituent of protein, the uptakes are probably merely too small to measure in this system. Table 1 depicts the results of a typical experiment.

Table 1. Arginine (per cent) remaining in Media after Incubation with Papilloma or with Hyperplastic Skin Epithelium

	Period of incubation 2 hr.	20 hr.
Papilloma	25	10
Hyperplastic skin	100*	110†

* Equivalent to 0·07 mgm. arginine per c.c.
† Increase from cellular breakdown.

As a result of the above findings, together with the fact that ornithine was regularly found in the media exposed to the papilloma tissue and not at all in the media exposed to skin, it was decided to find whether arginase could be demonstrated in extracts of papilloma tissue and in extracts of normal and hyperplastic skin. The arginase was extracted, concentrated, and tested by standard methods outlined elsewhere[2]. 3 gm. of papilloma tissue were ground to a paste with sand and an extract then made. The concentrated dialysed solution was activated by incubation at 37° C. for 3½ hr., and 0·5 ml. was then added to 0·5 ml. of a solution of arginine (7 mgm. per cent) in barbital buffer at *p*H 9·6 and incubated at 25° C. for intervals ranging from 15 min. to 20 hr. The mixture was then chromatographed and the amount of arginine determined comparatively. Hyperplastic and normal skin mixed in about equal proportion were extracted and concentrated in parallel. In the experiment, the results of which are shown in Table 2, 15 gm. of combined normal and hyperplastic skin were used. In no experiment has any detectable arginase activity been found in extracts of domestic rabbit skin derived from adults or embryos in the last trimester of gestation despite the fact that much larger amounts by weight have been tested than from the Shope virus-caused papillomas, which contain this enzyme in large amounts. A trace of arginase activity has been found in wild rabbit skin extracts. However, wild rabbit skin is extremely thin and the preparation contained an estimated 25 per cent of tissue other than skin epithelium.

Table 2. Comparative Arginase Activity of Extracts of Papilloma and of Normal and Hyperplastic Skin

	Arginine remaining after incubation period (per cent)		Ornithine after incubation (per cent)
	15 min.	20 hr.	
Papilloma extract	5·5	0	++++
Skin extract	120*	117*	0
Buffer plus arginine	100†	100	0

* Increase due to small amount of arginine present in concentrated dialysate.
† Equivalent to 0·07 mgm. arginine per c.c.

Only a trace of arginase activity has been detectable in a preparation of isolated and concentrated Shope virus containing 1·20 mgm. of protein nitrogen per c.c. incubated with 0·07 mgm. arginine per c.c. for 20 hr. The virus was isolated using the method of Beard, Bryan, and Wyckoff[3]. In view of the large amount of virus in this preparation, the arginase would seem most probably a contaminant rather than being an intrinsic attribute of the virus particles.

It is evident from these results that the Shope virus infection of epithelial cells is somehow inducing arginase in cells of a sort which contain no detectable arginase in their normal or hyperplastic state. One such means is that the presence of the virus in the cells induces or enables them to synthesize an enzyme new to them, arginase. Another possibility is that the virus induces a permease allowing the cells to concentrate arginine, the concentration of which in turn induces arginase. Either of these two possibilities would increase the metabolic efficiency of the cells. Might such an increase in efficiency be responsible for the overgrowth of these cells which is manifest as their neoplastic state?

This work has been supported by a grant from the Jane Coffin Childs Fund.

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Oral Induction of Leukæmia in Mice

The induction of leukæmia by parenteral inoculation of cell-free brain filtrates has been demonstrated[1,2]. Speculation as to the natural route of transmission of leukæmia led to the exploration of the leukæmogenic effect of whole brains and cell-free brain filtrates when given orally.

Swiss mice, approximately six weeks old, of both sexes, and *C3H* × 101 males were used. A 1 per cent concentration (w/v) of cell-free brain filtrates was prepared in the manner previously described[2], in which distilled water was used in place of physiological saline solution. The brain mince was prepared by forcing whole fresh brain through an 18-gauge needle with a syringe.

In experiment 1, Swiss mice were deprived of food and water for 24 hr. and then given cell-free brain filtrates as drinking water. Mice which received whole-brain mince were placed in individual cages and given approximately 0·5 gm. mince in a Petri

Table 1. EXPERIMENT 1

Material fed		No. leukæmic/No. fed
Brain mince	Non-leukæmic	0/25
	Leukæmic	4/25
Cell-free brain filtrate	Non-leukæmic	0/20
	Leukæmic	5/22

Table 2. EXPERIMENT 2

Material fed by intubation	No. leukæmic/No. fed
Non-leukæmic cell-free brain filtrate	0/25
Leukæmic cell-free brain filtrate	13/25

dish. Mice were allowed to eat or drink these materials for 24 hr. and then placed on regular diets. Results of this experiment are shown in Table 1.

In experiment 2 cell-free brain filtrates were prepared from the brains of *C3H* mice. *C3H* × 101 mice were lightly anæsthetized with ether. A No. 18 polyethylene tube was passed into their stomachs and through it 1 c.c. cell-free brain filtrate was injected. Results of this experiment are shown in Table 2.

In animals which developed leukæmia, the disease appeared within three to twelve weeks. Both the gross and microscopic results were similar to those previously described[1]. Identical leukæmias developed in seven out of ten mice inoculated intraperitoneally with 0·5 c.c. of the leukæmic cell-free brain filtrate used for feeding in experiment 1.

Brains of mice with orally induced leukæmia were later shown to be infective when the cell-free brain filtrate was given orally or intraperitoneally.

From the evidence presented, leukæmia in mice can be transmitted orally by either whole leukæmic brain or its cell-free filtrate. Investigators of leukæmia in mice should be aware of this possibility in the laboratory, where mice are caged together and where cannibalism occurs.

The influence of the gastrointestinal tract in the epidemiology of leukæmia remains to be elucidated.

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Experimental Colloid Goitre produced by Thiouracil

COLLOID goitres, resembling those occurring endemically in man, have been produced in the hamster by an iodine-deficient diet followed by repletion with this element after epithelial hyperplasia had developed[1]. It seemed possible that if proliferative activity of thyroid epithelial cells was produced in this species by other means, such as a goiterogen, colloid accumulation might occur when the stimulus was removed. Such would be in keeping with Marine's hypothesis of the pathogenesis of colloid goitre[2]. This has proved to be the case. The occurrence of colloid goitre in animals which had received thiouracil and allowed to recover from its effects may be helpful in our understanding of the pathogenesis of endemic goitre in man in cases where absolute iodine deficiency may not be a causative factor.

Hamsters weighing 100–125 gm. were fed diets of low iodine (2·5γ/100 gm.) or higher iodine (200γ/100 gm.) content which contained 0·3 per cent thiouracil. After subsisting on these regimens for varying periods of time up to 8 weeks the goiterogen was removed from the diet, after which the animals were allowed to live for varying intervals up to 2 months. At autopsy the thyroids were weighed and examined microscopically.

Extensive hyperplasia with loss of colloid accompanied by increased vascularity occurs within the first week after thiouracil administration. This progresses for the period during which this series of animals has been observed and conforms with the findings of others in the hamster[3,4]. The changes are more marked in animals on the low iodine intake. When the drug is removed from the diet, the follicles fill up with colloid and much of the epithelium becomes flattened, although sprigs of columnar cells are found projecting into the lumens. The degree of colloid deposition is related to the antecedent hyperplasia, that is, the time the animal received the goiterogen, together with the length of repletion. The follicles increase in size from their usual average of 45μ in diameter up to 100–150μ. The weight of the glands is increased tenfold or more.

The principal reason for this communication is to direct attention to the ease and rapidity with which diffuse colloid goitre may be produced by a goiterogen in the hamster. In our experience, comparable

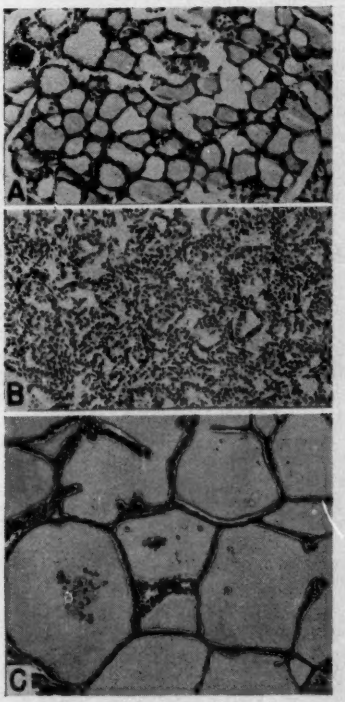

Fig. 1. Photomicrographs of thyroid glands. *A*, Normal; *B*, from an animal on low iodine–thiouracil-added diet for 20 days; *C*, from an animal comparable to *B* but off thiouracil for 3 weeks. (All × *c.* 84)

degrees of hyperplasia take a year to develop on an iodine-deficient diet[1].

Marine[5] expressed the belief fifty years ago that goitre was an iodine-deficiency disease. He viewed its pathogenesis as follows: iodine deficiency leads to hyperplasia which continues unless this element is administered; whereupon the gland fills up with excess colloid, a state representing "the nearest approach to normal glands that active hyperplasias can become". The role of the pituitary in this process is, of course, now recognized. The main difficulty in accepting Marine's hypothesis in its entirety has been that the structural alterations seen in the naturally occurring disease had not been produced in laboratory animals[6]. This is particularly true with respect to diffuse colloid goitre, although few investigators have tried to put Marine's hypothesis to the test. Most recent pathological studies on the thyroid have dealt with tumour production. Mackenzie[7] has summarized the story of experimental goitre with particular respect to the effects of iodine deficiency and goiterogenic agents, and points out that "with the judicious use of antithyroid drugs and thyroxine (or perhaps diet) we may be able to produce the elusive colloid goitre in the laboratory". Since this has been accomplished by iodine deficiency and goiterogens in the hamster it appears that pointed studies of other species, excepting perhaps the rat[7], will be of interest and throw further light on the pathogenesis of endemic goitre in man.

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Variability of Content of Deoxyribonucleic Acid in L-strain Fibroblasts

It is well known and has been amply demonstrated that the deoxyribonucleic acid content of normal 'resting' mammalian cells in the host is constant from cell to cell and tissue to tissue. The germinal tissue is the single exception, having instead exactly half the quantity of acid. This has been felt to reflect the constant chromosome number of these cells.

In tissue-culture cells, however, conflicting evidence is at hand. Some work apparently demonstrates a constant level of deoxyribonucleic acid, while other investigations reveal changing content of the acid depending on the cultural circumstances. Simonovitch *et al.*[1] found that in their rotating suspension cultures of *L*-strain fibroblasts, the deoxyribonucleic acid content paralleled at all times the cell number. Their cultures were always seeded from a parent culture which was in the logarithmic phase of growth. Recently, Salzman has used HeLa cells to obtain different results. He finds that during the lag phase of these cells grown as monolayers on glass, there is doubling of deoxyribonucleic acid, ribonucleic acid and protein, while the cell number does not change. On the other hand, when these cells are grown in suspension cultures, ribonucleic acid and protein but not deoxyribonucleic acid rise before the number of cells[2].

In our work with *L*-strain fibroblasts in continuous agitation suspension cultures[3], we have made some observations which incorporate these apparently conflicting data. Fig. 1 illustrates the fact that, when a subculture is made from a parent culture which is old and in the stationary phase (reflected in a stable cell population and low values of protein per cell), the content of deoxyribonucleic acid per million cells in the subculture follows a sharply rising and falling curve: during the first day, the deoxyribonucleic acid almost doubles, while cell population remains unchanged. When, on the other hand, the subculture is seeded from a parent culture which is in logarithmic growth at the time of the transplant, the daughter culture immediately goes into logarithmic growth, and content of deoxyribonucleic acid per million cells remains constant throughout the log growth period. Protein content, also shown in Fig. 1, parallels the content of deoxyribonucleic acid in both instances. A lag phase can be produced at will in any culture by adjusting initial *p*H to 7·4.

If the constant deoxyribonucleic acid level of the cells seeded from cultures in logarithmic growth represents the basic chromosomal acid-level of these cells, then it is clear that the cells seeded from stationary cultures replicate deoxyribonucleic acid to almost twice the normal complement in the 24-hr. lag phase. Apparently in these latter cells, the stimuli for replication of deoxyribonucleic acid and for division have become disordered and out of phase during the stationary period, with the result that while replication of deoxyribonucleic acid proceeds, division does not. In most normal cells, it seems clear that replication of deoxyribonucleic acid occurs during the last half of interphase[4], and if these cells were synchronous in time of division, it would appear that the accumulation of deoxyribonucleic acid merely represents this pre-mitotic replication. However, multiple mitotic counts using aceto-orcein stain have demonstrated no evidence of such synchrony in our cultures. Furthermore, the cell count increases gradually in the first 24 hr. of logarithmic growth.

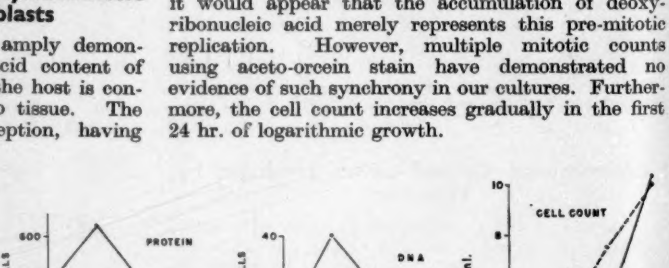

Fig. 1. Protein, deoxyribonucleic acid and cell count in fibroblast cultures. The solid lines refer to cultures seeded from parent cultures in stationary phase; the broken lines refer to cultures seeded from parent cultures in log growth phase. Each line represents the average of at least four experiments. Deoxyribonucleic acid was determined by the indole method (ref. 5), protein with phenol reagent (ref. 6) and cell number was counted in a haemacytometer on aliquots of the undiluted medium

The results presented bring together some of the previous apparently conflicting data, and clearly support Salzman's contention[2] that deoxyribonucleic acid and protein content cannot be used indiscriminately as indications of cell number in tissue culture.

This work was supported by a grant from the United States Public Health Service C2928 (C2).

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Stress Activation of Adrenal Glomerulosa

THE experimental data concerning the hormonal regulation of adrenal glomerulosa and its mineralocorticoid production[1] are still inconclusive. The demonstrated dependence of the glomerulosa reaction on the presence of pituitary[2] as well as on adrenocorticotrophic hormone[3] is contradictory to the absence of increased aldosterone secretion by adrenals perfused with either adrenocorticotrophic or somatistrophic hormone *in vitro*[4]. Nevertheless, it can be assumed that the former, and stress, activate the adrenal glomerulosa indirectly[5], mediated by the pituitary-dependent glucocorticoid production, which becomes properly balanced by means of mineralocorticoids[6]. This view will be supplemented by further experimental results.

Adult Wistar rats, males or females, divided into three separate groups (96 animals altogether), were subjected to stress by a single intra-muscular injection of 0·3 ml. 4 per cent formaldehyde/100 gm. body-weight. At intervals of 3, 6, 12, 24, and 48 hr. rats were killed. Frozen sections of adrenals were stained with sudan black *B* and with oil red *O* on lipids. The width of the zona glomerulosa was measured at a magnification of × 330, in all four quarters of the adrenal cortex. The number of leucocytes, eosinophils, and the differential count of leucocytes were also determined; the spleen and thymus were examined histologically.

In the unstressed rats of all three series the lipid content of the zona fasciculata was relatively low, resembling that of series *B*, described previously[5]. 3–6 hr. after the formaldehyde injection a considerable decrease and even disappearance of lipids took place in the fasciculata. The lipid content of the glomerulosa, however, remained unchanged. 24–48 hr. after the injection the lipids of fasciculata were gradually restored and simultaneously the glomerulosa was activated, as judged from the depletion of their lipids and from a statistically highly significant ($P<0·001$) enlargement (Fig. 2). The glomerulosa activation was associated with the transformation of light centres of spleen lymphatic nodules, previously

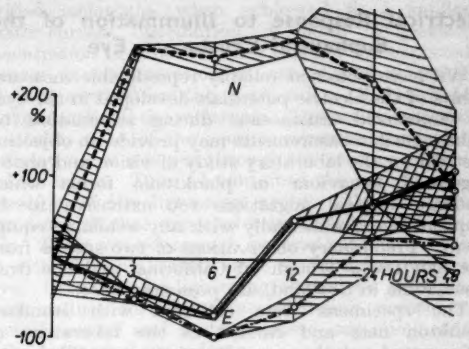

Fig. 1. The stress changes of neutrophils (*N*), lymphocytes (*L*) and eosinophils (*E*) expressed as a percentage of the mean initial value, with their standard deviations

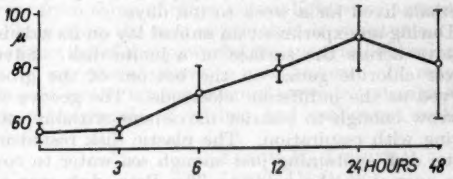

Fig. 2. The stress changes of the mean width of the zona glomerulosa in μ and their standard deviations

activated by stress, into resting ones, and with the return of neutrophils to the initial level; that of lymphocytes ($P<0·01$) and eosinophils rose above the initial level (Fig. 1).

The findings show that the activation of the glomerulosa corresponds to changes in the leucocytes and the lymphoid tissue opposite to those found during the glucocorticoid phase of stress. Taking into account the mutual antagonism between mineralocorticoids and glucocorticoids[6], we may suppose that the activation of the glomerulosa is elicited by the antecedent glucocorticoid effects, especially their influence on electrolyte and water exchanges through the cell wall. Hence the stress reaction of the glomerulosa occurs as a balance to the glucocorticoid effects by abolishing them. The stress period can thus be divided into well-defined glucocorticoid and mineralocorticoid phases.

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Electrical Response to Illumination of the Euphausiid Crustacean Eye

We have obtained reliably reproducible measurements of the electric potentials developed in the eyes of euphausiid crustaceans during stimulation by light. Such measurements may provide an objective method for the laboratory study of vision and photoregulated behaviour in planktonic forms which undergo vertical migrations too extensive to be duplicated experimentally with any available equipment. Preliminary observations of two species from the San Diego Trough off California, and one from Loch Fyne in Scotland, are presented here.

The specimens were collected with standard plankton nets and carried to the laboratory in refrigerated polythene or glass containers filled with off-shore water. Low temperatures (2–9° C.) and darkness improved their chance of survival, and, after an initial 24-hr. recovery period, any surviving animals lived for a week to ten days.

During an experiment an animal lay on its side in a groove across the surface of a lucite disk. Silver–silver chloride gauze on the bottom of the groove served as the indifferent electrode. The groove was narrow enough to restrict movement without interfering with respiration. The plastic disk rested in a Petri dish containing just enough sea water to cover the animal in the groove. The Petri dish was surrounded by an ice bath.

The recording electrode was a piece of tungsten wire etched electrochemically to a tip of less than 2-μ diameter, and insulated down to near this tip with 'Insulex'. (The electrode was dipped in a diluted solution of 'Insulex', clamped with tip upward and dried rapidly in a gentle downward air current.) This microelectrode was attached to a plastic rod held in a ball-and-socket joint, such that the tip might penetrate the eye without blocking the light. Glass micropipettes with silver–silver chloride electrodes were used at first, but penetration of the eye with the tungsten electrodes seemed to give less damage as judged by survival time and gave identical electrical responses. The preparation was housed in a light-tight, copper-shielded box.

The light source, a 12-volt concentrated tungsten filament lamp, was situated outside the box, as were the filter holder and shutter, so that colour, intensity and duration of the stimulating flash could be altered without disturbing the preparation. The light entered the box through a baffled collimating tube, struck a concave mirror set at 45° across the light path, and was directed downward on to the exposed eye. Interference filters were used in the colour determinations, and the dimensions of the collimator were such that the useful 5° cone of these filters was not exceeded. A heavy-duty storage battery supplied power for the lamp, and the voltage across the lamp terminals was monitored throughout the experiments. Light intensity was varied with neutral density filters.

The inner surfaces of the lamp housing, filter holder, collimator and light-tight box were painted matt black.

Electrical signals from the recording electrode were fed via a cathode follower to a resistance-coupled amplifier and an ink writer or a cathode-ray oscilloscope. Control experiments were run to make sure that no artefact occurred due to photochemical properties of the electrode system. No electrical signals were detectable when the recording electrode was illuminated at the highest experimental intensity, whether simply immersed in sea water or inserted in the eye of a dead animal. Furthermore, in the absence of light on the eye, no electrical activity could be detected even when the animal exerted swimming movements.

At the outset of an experiment the room was darkened, the stimulating lamp was turned on, the shutter was opened and a deep red filter was placed in the light path. The animal was transferred quickly from the dark storage vessel to the indifferent electrode. The position of the Petri dish was adjusted such that only the eye of the subject was illuminated, and the recording electrode was inserted. The shutter and the door of the light-tight box were then closed, and the animal remained in total darkness for at least 30 min. before measurements were begun.

The response to a brief flash (100 msec.) of 'white' light of moderate intensity is shown in Fig. 1, with representative curves from (*A*) *Euphausia pacifica*, (*B*) *Nematoscelis difficilis* and (*C*) *Meganyctiphanes norvegica*. The response in each species is rapid, with a small *a*-wave lasting about 20 msec., a main response which reaches a peak in 40–50 msec., and a final overshoot in the other direction. Over a defined range of intensities the height of the main response to short flashes of light varies directly with the logarithm of the intensity of the light. At very low intensities the signal is lost in the noise, and at high intensities the signal reaches a limiting value, of the order of 200–300 μvolts.

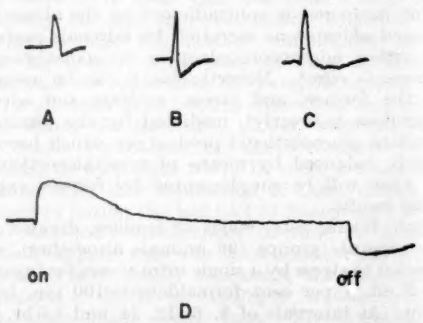

Fig. 1. Response to a 100-msec. flash of 'white' light by: (*A*) *Euphausia pacifica* (recorded with a cathode-ray oscilloscope); (*B*) *Nematoscelis difficilis* (recorded with a Beck–Lee ink writer); (*C*) *Meganyctiphanes norvegica* (recorded with an electro-encephalograph designed by Dr. Grey Walter); (*D*) response of *M. norvegica* to a 5-sec. flash

Light flashes of longer duration induce an electrical response at both on and off (Fig. 1 *D*).

Preliminary studies of response to light of various wave-lengths have been made, both for equal biological response and for equal energy of illumination. The euphausiid eye is most sensitive to blue-green light; but the spectral sensitivity curve does not coincide exactly with the spectral absorption curve of euphausiopsin, the light-sensitive pigment reported from the eyes of *Euphausia pacifica*[1].

The work on *E. pacifica* and *N. difficilis* was done at the Scripps Institution of Oceanography. The work on *M. norvegica* was done at The Laboratory, Plymouth. We should like to thank Dr. F. S. Russell, of Plymouth, and Dr. Harold Barnes, of Millport, for making the latter possible.

This work was carried out by the University of California under contract with the U.S. Office of Naval Research. Additional support was provided by National Science Foundation grant G–4801.

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Melatonin in Peripheral Nerve

MELATONIN is a hormone, isolated from beef pineal glands, that lightens the colour of frog melanocytes either after injection into the frog or after *in vitro* incubation with pieces of frog skin[1]. In addition, this hormone can block and reverse the darkening actions of the melanocyte-stimulating hormones and also of adrenocorticotrophic hormone. Melatonin is a 5-hydroxyindole derivative in which the hydroxy group is blocked[2]. Its melanocyte-lightening properties are more than ten thousand times greater than those of noradrenaline, which previously was considered to be the most potent lightening agent known[3]. In our early experiments melatonin could be detected only in extracts from pineal glands and not from other tissues. With improvement in extraction techniques we have been able to identify the presence of a lightening agent that is most likely melatonin in peripheral nerves of man, monkey and cow. A detailed mapping of the location of melatonin in the nervous system has not been completed.

Sciatic nerves from human beings were collected at autopsy and stored at −10° C. until a total of 65 gm. of tissue was obtained. This material was lyophilized to give 28 gm. of dry tissue. The latter was cut into small pieces and defatted with petroleum ether in a Soxhlet extractor. 10 gm. of dry, defatted nervous tissue was obtained which was pulverized to a powder in a Waring blendor. The powder was mixed with 100 ml. water in the blendor, and the mixture was centrifuged at 15,000*g* for 30 min. The supernatant fluid was filtered and the residue extracted with 100 ml. water and centrifuged again. The combined filtrates were extracted twice with equal volumes of ethyl acetate. The ethyl acetate layer was evaporated to dryness *in vacuo*. The residue was dissolved in a mixture of 10 ml. of each layer of solution used for counter-current distribution. This solvent system was made by equilibrating 2 parts of water with 1 part of ethylacetate and 1 part of *n*-heptane. All solvents were redistilled prior to use. The dissolved residue was subjected to 29 transfers in a counter-current distribution apparatus. The contents of the tubes were pooled into four groups: 0–7, 8–14, 15–22 and 23–29. From each pooled group the organic phase plus the upper phase obtained by re-extraction of the water layer with an equal volume of ethyl acetate were combined and evaporated to dryness *in vacuo*. The four residues, each dissolved in 2 ml. ethanol, were assayed for their ability to lighten isolated pieces of frog skin. Activity was found only in material from tubes 8–14. Similar results were obtained from extracts made in the same way using collections of sciatic, femoral and brachial nerves of monkeys and spinal nerve roots of cows. Purified melatonin, when subjected to a similar counter-current distribution, is found in greatest concentration in tube 11; and there is the expected decrease in concentration as one moves in either direction away from the peak tube. Tubes 8–14 contain nearly all the melatonin. The active material from tubes 8–14 of the counter-current distribution of human nerve was put through the counter-current machine again. Tube 11 contained the highest concentration of the lightening agent. This location coincides with that expected from distributing melatonin. There was not enough material in tubes 8–14 to test with Ehrlich's reagent or to obtain a fluorescence curve. Other known lightening agents, for example, noradrenaline, adrenaline, acetylcholine, serotonin, triiodothyronine and hydrocortisone, are eliminated by the extraction procedures.

Since the solubility and biological properties of the agent obtained from nerves of human beings, monkeys and cows are identical with those of melatonin and different from those of other known lightening agents, it is likely that the active principle is melatonin.

The function of this powerful melanocyte-lightening agent in peripheral nerve is not known. It may play a part in the transmission of nerve impulses and in the pathogenesis of vitiligo and malignant melanomas.

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Effect of Enzymes on Mechanical Properties of Tissues

WHILE investigating the effect of extending forces on the excised uterine cervix of the rat[1] we found that at the end of pregnancy the tissue showed prolonged extension at constant rate under low loads, behaving as if the collagenous framework in it was not continuous, as it is in the majority of tissues, but was one in which the ultimate links were viscous. In the course of investigating this viscous state we tried the effect of soaking the tissue in solutions containing trypsin. This was found to increase dramatically the rate of extension under constant load. Similar results were obtained from rings of skin cut from around the body-wall of newly born rats. Chymotrypsin was also tried on these and found to have the same effect as trypsin.

After excision, tissues were left overnight in the refrigerator (5° C.) in the enzyme solutions (crystalline Armour preparations, trypsin 0·5 mgm./ml., chymotrypsin 1·0 mgm./ml.) and incubated for 15 min. at 37° C. before testing. The method of using the enzymes and the concentration of trypsin were suggested to us by P. B. Medawar as suitable for detaching epidermis without killing the cells. Tissues were stretched between two parallel steel rods (stainless steel hypodermic needle tubing 0·6 mm. diam.). In the case of the cervix, one rod went through each canal, while the skin formed a belt around both rods.

Table 1. Rate of Linear Extension (percentage of l_0 per min.) under Constant Load
The number of animals is given in brackets. The estimate of variation is the standard error of the mean

	Normal	Trypsin-treated	Chymotrypsin-treated
Cervix (twenty-first day pregnancy) 50 gm. load	1·37 ± 0·33 (6)	30·7 ± 6·2 (3)	—
Skin (new born) 12 gm. load	0·038 ± 0·004 (6)	39·3 ± 10·2 (3)	4·7 ± 1·1 (3)

The tests were made in oxygenated Locke's solution, the tissues being loaded for periods up to 1 hr. and then broken by increasing the load in steps at constant intervals if they had not broken before.

Quantitative results are given in Table 1. The rate of extension under load is given as a percentage of the length at zero time (l_0) obtained by extrapolation backwards of the linear part of the curve (Fig. 1). Tension per unit cross sectional area of tissue calculated from weight of tissue for length l_0 was about 3 gm./mm.^{2} for both cervix and skin; per unit cross sectional area of collagen it was lower in the more collagenous cervix, that is, about 50 gm./mm.^{2} collagen as compared with about 200 gm./mm.^{2} collagen for skin.

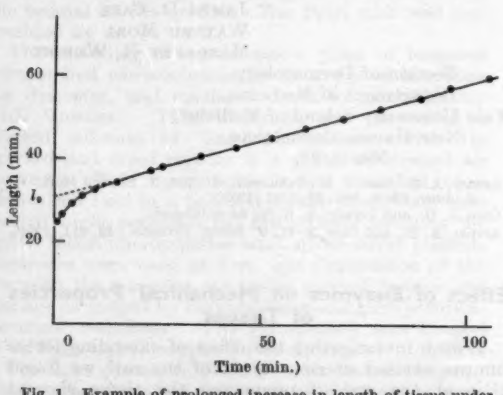

Fig. 1. Example of prolonged increase in length of tissue under constant load in cervix of rat at the end of pregnancy (twenty-first day). Length on the ordinate is that of the circumference of tissue around the rods. Load was 50 gm., giving a tension of approximately 3 gm./mm.^{2} at l_0

In contrast with the above results we have been unable so far to find any appreciable effect with hyaluronidase ('Hyalase', Benger).

Collagen itself is not attacked by trypsin or chymotrypsin, and these experiments appear to provide strong evidence that the collagenous framework is held together by some other unidentified protein on which the mechanical properties and integrity of the whole tissue depends. They strongly support the views put forward by Day[2] on the nature of the so-called 'cement substance' in connective tissue.

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Hydrolysis of Lecithin and Lysolecithin by Rumen Micro-organisms of the Sheep

Although the diet of the ruminant contains only a small percentage of complex lipids (triglycerides, phospholipids, sterols, waxes), the actual amount ingested can be considerable because of the bulk of the food eaten. Furthermore the surface activity of such lipids may play an important physico-chemical part in ruminant digestion, and recent evidence suggests that the ingested chloroplast lipids act as efficient anti-foaming agents in the rumen[1]. At the present time, little is known about the way in which these complex lipids are digested by ruminants. Recently, however, Garton, Hobson and Lough[2] have described a rapid hydrolysis of triglycerides when these were incubated with sheep rumen contents.

The present communication describes observations made over the past two years on the hydrolysis of phospholipids by sheep rumen micro-organisms. When lecithin labelled with phosphorus-32[3] was incubated with sheep rumen contents, or a washed preparation of rumen micro-organisms there was a rapid breakdown of lecithin and the phosphorus-32 label appeared in the water-soluble fraction mainly as inorganic phosphorus. Similar incubations have been performed in the presence of a series of likely metabolic intermediates of lecithin hydrolysis, for example, phosphorylcholine, glycerylphosphorylcholine, glycerophosphoric acid, in order to trap the radioactive fragment split off from the lecithin and largely protect it from further catabolism. These substances were then separated from other phosphorus compounds in the incubation medium by two-dimensional paper chromatography and their radioactivity measured both by autoradiography and also by direct radioactive assay of the digested spots. Such experiments showed that the major catabolic pathway was via glycerylphosphorylcholine and glycerophosphoric acid. At the same time there appeared to be a minor pathway via phosphorylcholine.

Washed preparations of rumen micro-organsims also hydrolysed lysolecithin, although individual animals showed wide variations in the level of activity. By autolysis of the micro-organisms or on their treatment with butanol a soluble preparation of the enzyme was obtained. This has been purified by ammonium sulphate precipitation and adsorption on calcium phosphate gel. Such preparations possess no lecithinase activity but they readily hydrolyse lysolecithin producing glycerylphosphorylcholine. Lysophosphatidyl ethanolamine is also attacked. The *p*H optimum in collidine–hydrochloric acid buffer is 7·5, which is different from the *p*H optima of the lysolecithinases of animal tissues (6·0) and moulds (3·3–4·0). In buffers containing alkali metals, for example sodium glycine–glycine, the activity was much greater at high *p*H's (7·5–9·5) than in buffers with organic bases. The enzyme does not appear to need any metal ion for activation, and it is not appreciably affected by ethylenediamine tetraacetic acid. It is comparatively insensitive to inhibition although copper and zinc ions are effective, as is also 0·01 per cent cetyltrimethylammonium bromide.

No information has been obtained as to the identity of the organism(s) responsible for the phospholipid hydrolysis. Cultures of mixed rumen streptococci grown in a glucose rumen–liquor medium possessed little lysolecithinase activity. Hayaishi and Kornberg[4] have reported that a soil bacterium *Serratia plymuthicum* can metabolize lecithin via glycerylphos-

phorylcholine while the hydrolysis of lecithin to phosphorylcholine (lecithinase *C*) by some members of the *Clostridium* genus is well known[5,6]. Recently, *Bacillus cereus* has also been shown to contain a lecithinase *C*[7].

The level of lysolecithinase in the rumen micro-organisms seems to be dependent upon the diet of the animal. Nevertheless the presence of phospholipid in the food did not appear to be essential, as an animal receiving a synthetic diet of cellulose and mineral salts with a minimum hay supplement (15 per cent) still retained considerable lysolecithinase in its rumen micro-organisms. It is interesting, however, that in these circumstances the enzyme was no longer solubilized by autolysis of the micro-organisms although butanol treatment was effective.

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Bilirubin Metabolism in the Fœtus

It has been shown that in fœtal liver the enzymatic mechanism for glucuronide formation is poorly developed, while a rapid increase in enzymatic activity occurs during the early neonatal period[1]. The transient hyperbilirubinæmia observed during the first few days of life is believed to be related to this inadequate development of the hepatic conjugating system[1]. Since a significant increase in serum bilirubin concentration usually occurs only after the cord is severed, it has been assumed that during gestation the placenta is responsible for the removal of fœtal bilirubin. It has not been determined, however, whether the placenta has the ability to conjugate the fœtal pigment or whether placental transfer of unconjugated bilirubin occurs from the fœtal to the maternal circulation.

In vitro, glucuronide formation was studied[2] both with human placenta obtained at term or by Cæsarean section and with rat placenta at various stages of gestation. Incubations were carried out with tissue slices, homogenates and microsomal preparations in the presence of UDPGA, using as substrates bilirubin, *o*-aminophenol and *p*-nitrophenol[2]. In these systems, no evidence for glucuronide formation was obtained. Moreover, supravital perfusion of human placenta[3] failed to reveal formation of bilirubin glucuronide.

In view of these negative results, placental transfer of bilirubin was studied in pregnant guinea pigs during the last week of gestation using a modification of the method described by Dancis *et al.*[4]. On the maternal side, a uterine vein was cannulated which permitted continuous sampling of venous blood from an individual placenta. The corresponding fœtus was delivered into a saline bath, maintained at 37° C., and an umbilical artery was cannulated, while the umbilical vein was severed and the cord clamped proximal to the fœtus. Over a period of 1 min., 0·4 mgm. crystalline bilirubin, dissolved in 1 ml. of either 5 per cent human albumin or guinea pig plasma, was infused through the cannulated umbilical artery. In addition, the injected solution contained either iodinated human albumin (2,500,000 counts/min./ml.) or chromated guinea pig erythrocytes (3,000,000 counts/min./ml.). Blood samples from the uterine vein were taken before, during and at various time-intervals after the injection for estimation and paper chromatography[5] of bilirubin and for determination of the radioactivity in albumin and in erythrocytes.

The injected bilirubin was rapidly transferred through the placenta and was present in the maternal blood as non-conjugated bilirubin. Peak concentrations ranging from 0·6 to 2·0 mgm. per cent were reached in the uterine venous blood 2–4 min. after the beginning of the injection. As seen in Fig. 1, iodinated albumin was transferred at a rate similar to that of bilirubin. On the other hand, no radioactivity was detected in the maternal blood if bilirubin was injected together with chromated erythrocytes. Similarly, injection of crystalline bilirubin into the maternal circulation resulted in its rapid appearance in the fœtal blood. Conversely, if bilirubin glucuronide obtained from human bile and iodinated albumin were injected into the umbilical artery, transfer of conjugated bilirubin was minimal, while radioactive albumin rapidly appeared in the maternal blood.

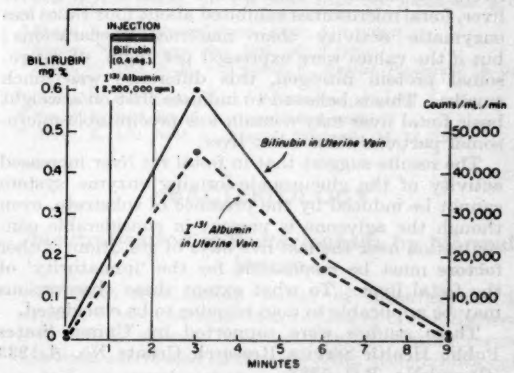

Fig. 1. In a pregnant guinea pig shortly before term, 0·4 mgm. bilirubin and albumin labelled with iodine-131 (2,500,000 c.p.m.) were injected into a fœtal umbilical artery. Concentrations of bilirubin and radioactive albumin were determined in the corresponding uterine vein

These observations indicate that under the experimental conditions employed, non-conjugated bilirubin was rapidly transferred from the fœtal to the maternal circulation, while transfer of conjugated bilirubin was insignificant. They are in agreement with the findings of Dancis *et al.*[4] that œstrogens but probably not œstrogen glucuronides can pass the placental barrier. In view of these findings, the possibility was considered that the inadequate development of the mechanism for glucuronide formation in the fœtal liver may be due to a lack of substrate during the intra-uterine period. This problem was studied by injecting six pregnant rats with large amounts of anthranilic acid, a compound which is largely excreted as an acyl-glucuronide[6]. During the last six days of gestation, 50 mgm. sodium anthranilate/100 gm. body-weight were injected twice a day by intraperitoneal route and a

Table 1. Effect of Injection of Sodium Anthranilate on the Biosynthesis of *o*-Aminophenyl Glucuronide by Microsomes of Maternal and Fœtal Rat Livers *in vitro*

Type of treatment and No. of animals	*o*-Aminophenyl glucuronide formed	
	Mother	Fœtus
Saline (control), 6	0·044 ± 0·013	0·008 ± 0·005
Anthranilate, 6	0·041 ± 0·019	0·011 ± 0·003

Washed microsomes obtained from 1 gm. of liver were incubated for 30 min. at 37° C. with 0·2 μmole *o*-aminophenol, 1 μmole UDPGA and 25 μmoles magnesium chloride in 0·1 *M tris* (hydroxymethyl) amino-methane buffer, *p*H 8·0. Results are expressed as μmoles formed per gram of liver ± standard deviation

similar group of pregnant rats was given a comparable amount of saline. 12 hr. after the last injection, the animals were decapitated, and microsomes were prepared from the fœtal and maternal livers. In mothers treated with anthranilate, fœtal liver exhibited anthranilic acid concentrations[7] of 18·3 μgm. (*S.D.* 5·9 μgm.) per gm. tissue, while none was detectable in maternal livers or in fœtal livers of saline-treated mothers. Washed microsomes obtained from 1 gm. of liver were incubated with *o*-aminophenol and UDPGA and the amount of *o*-aminophenyl glucuronide formed was estimated[2]. As seen in Table 1, no difference in glucuronide formation was observed between microsomal preparations of fœtal livers that were obtained from mothers treated with anthranilate or with saline. Moreover, the magnitude of glucuronide formation by microsomes of maternal livers was similar in both groups of rats. Per gm. of liver, fœtal microsomes exhibited about four times less enzymatic activity than maternal preparations; but if the values were expressed per mgm. of microsomal protein nitrogen, this difference was much smaller. This is believed to indicate that on a weight basis fœtal liver may contain less precipitable microsomal particles than adult liver.

The results suggest that in fœtal rat liver increased activity of the glucuronide-forming enzyme system cannot be induced by the presence of substrate, even though the aglycone is present in considerable concentration over the last five days of gestation. Other factors must be responsible for the 'immaturity' of the fœtal liver. To what extent these observations may be applicable to man remains to be elucidated.

These studies were supported by United States Public Health Service Research Grants No. *A*-1833 (*C*) and No. *R.G.*-5333.

We gratefully acknowledge the help of Dr. P. Troen[3].

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Flavone Glucosiduronic Acids in *Scutellaria* sp.: a Correction

In the course of studies on the isolation of flavone glucosiduronic acids from various skull-caps, specimens of a localized colony at Mells, Somerset, which had been identified as *Scutellaria columnae* Tenore[1], were reported[2] to contain scutellarin (5,6,7,4′-tetrahydroxyflavone β-glucosiduronic acid) and not baicalin (5,6,7-trihydroxyflavone β-glucosiduronic acid) as found previously[3] in this species grown in the area of Paris. This anomaly is now removed by the recent re-identification[4] of the Mells skull-cap as *Scutellaria altissima* L., a native of south-east Europe. Scutellarin was originally isolated from this plant[5].

Thanks are due to Dr. A. J. Willis for directing my attention to this reclassification.

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Relationship between Auxins and Nucleic Acid Synthesis in Coleoptile Tissues

That auxins may affect nucleic acid metabolism in plants has been suggested by a number of workers. Silberger and Skoog[1] had observed an increase in both the deoxyribonucleic acid and ribonucleic acid content of tobacco pith cells incubated with 0·01 to 10 mgm./l. of indole acetic acid. Growth continued in a manner somewhat similar to changes in the ribonucleic acid content for a few days even though the synthesis of nucleic acids had apparently stopped after four days of application of indole acetic acid. Holmes *et al.*[2] have shown that in *Vicia* root, as the distance from the root tip increased the content of deoxyribonucleic acid per cell also increased. Attempts have been made to correlate root growth with nucleic acid metabolism in other plants as well[3–5]. In maize roots it has been found that application of indole acetic acid resulted in a slowing down of growth accompanied by a decrease of both deoxyribonucleic acid and ribonucleic acid[3]. Some workers are inclined to believe that growth and synthesis of deoxyribonucleic acid in plants continues only in the presence of auxins[6–8]. The present communication summarizes the results obtained in an investigation on the incorporation of compounds labelled with phosphorus-32 and carbon-14 into the coleoptile tissues as affected by indole acetic acid.

Seeds of *Avena sativa* var. *N.P.*1 and *Oryza sativa* var. Rupsail were used. The seeds were germinated in the dark with occasional red light for 3 days. The coleoptiles were then decapitated at 2–3 mm. and floated on water for 2 hr. to free them from any endogenous auxin. After a further decapitation by another 2–3 mm., 5-mm. sections were cut off and floated on the experimental solution containing $Na_2H^{32}PO_4$ (50 μc./ml.) of specific activity greater than 1 mc./μgm. phosphorus in *M*/150 phosphate buffer of *p*H 6·0 for 2 hr. with or without indole acetic acid. For rice, 5-day old coleoptiles were used. ($Na_2H^{32}PO_4$ was obtained from N.V. Philips Roxane

Table 1. The Effect of Indole Acetic Acid on Incorporation of Phosphorus-32 in the Nucleic Acids of Rice (var. Rupsail) Coleoptiles

Results expressed as counts/min. in the nucleic acid per mgm. of coleoptile × 10^{-3}

Treatment	Ribonucleic acid	Deoxyribonucleic acid
Control	426·6	296·5
10^{-6} *M* indole acetic acid	632·6	387·4

Pharm.-Chem. Industrie Netherlands.) After the end of the incubation period, cold trichloracetic acid was added and the nucleic acids fractionated according to the method of Ogur and Rosen[9]. Measurements of radioactivity were taken with a Nuclear Chicago halogen counter coupled with a Tracerlab autoscaler.

The results are presented graphically in Fig. 1. With low concentration of indole acetic acid there is a marked stimulation of incorporation of phosphorus-32 into both deoxyribonucleic and ribonucleic acid fractions. In the latter, a concentration of 10^{-9} *M* indole acetic acid has practically no effect; but 10^{-6} *M* has a distinct stimulatory effect, the increase observed being more than 50 per cent. In the former, the rate of incorporation of phosphorus-32 is much slower, and the maximum increase observed due to the application of indole acetic acid was 20 per cent. With 10^{-3} *M* indole acetic acid there appears to be an inhibition in the incorporation of phosphorus into deoxyribonucleic acid. Similar stimulation of incorporation of phosphorus-32 due to low concentrations of indole acetic acid was also observed when rice coleoptiles were incubated with 125 μc./ml. of $Na_2H^{32}PO_4$ in phosphate buffer with and without 10^{-6} *M* indole acetic acid.

To study the utilization of compounds labelled with carbon-14 for nucleic acid synthesis the coleoptiles were incubated with the following compounds (obtained from the Radiochemical Centre, Amersham, Great Britain) in *M*/165 phosphate buffer of *p*H 7·0 with or without 10^{-6} *M* indole acetic acid: (1) sodium acetate-1-^{14}C (37 μgm./μc.)—45 μc./ml.; (2) acetate-2-^{14}C (14·9 μgm./μc.)—9 μc./ml.; (3) $NaH^{14}CO_3$ (3·7 μgm./μc.)—45 μc./ml.; (4) sodium formate-^{14}C (14·1 μgm./μc.)—45 μc./ml.; (5) glycine-1-^{14}C (22 μgm./μc.)—10 μc./ml. When the nucleic acids were extracted and isolated as described above no stimulation of incorporation of carbon-14 was observed in both ribonucleic and deoxyribonucleic acids, with the exception of glycine.

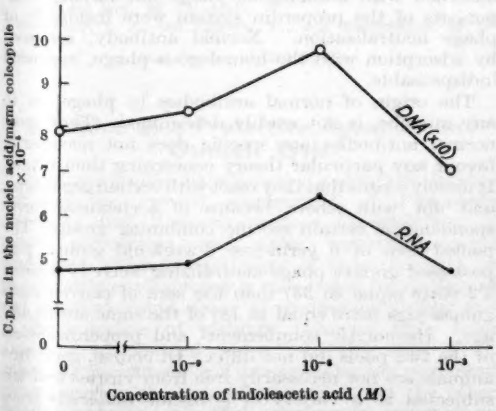

Fig. 1. Effect of indole acetic acid on the incorporation of phosphorus-32 into the nucleic acids of *Avena* coleoptile. The values for deoxyribonucleic acid have been multiplied by 10 for clarity

It thus appears that the effect of auxins on nucleic acid synthesis during the first 2 hr. of application is exercised through its effects on the synthesis of nucleotides composing the nucleic acids. That indole acetic acid stimulates the synthesis of nucleotides and phosphorylation reactions has now been confirmed with compounds[10] labelled with phosphorus-32, as also through the recent work of Marre[11]. The compounds labelled with carbon-14 used are all known to contribute to the synthesis of purine and pyrimidine bases of the nucleic acids. Apparently during the first 2 hr. of application of indole acetic acid the reactions leading to their synthesis or of the sugar moieties of the nucleic acids are not much affected, even though the phosphorylation reactions are.

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BACTERIOLOGY

Neutralization of Bacteriophage by Normal Serum

Normal mammalian serum of most species is capable of neutralizing bacteriophages of the *Escherichia coli T* series. This activity of normal serum has been attributed to the properdin system although a lack of correlation between properdin-levels and phage-neutralizing activity was noted with certain sera[1]. Recent work by Cowan[2], moreover, in which several human sera were assayed for activity against *T2*, *T6*, and *T7*, indicated that the phage-neutralizing activity of a serum against one of the phage types was not correlated with its activity against the other two. These results suggested that the properdin system, essentially non-specific in its action, could not adequately account for the activity of normal serum against the three phage types. Cowan postulated, therefore, that properdin might be a family of cross-reacting antibodies capable of combining with zymosan. Similar considerations are applicable to other activities of normal serum. Our own findings in studies of the bactericidal action of normal serum against several species of Enterobacteriaceae indicated that bactericidal antibody of marked specificity plays the major part[3].

A more direct demonstration of the significance of normal antibody of marked specificity, as opposed to properdin, in the phage-neutralizing activity of normal serum was obtained in our laboratory.

Escherichia coli bacteriophage *T2* was prepared and concentrated as described by Herriot and Barlow[4]. High-titred cultures of phage *T5* were obtained by using the procedure outlined by Adams[5]. Phage-*E. coli* complexes were prepared by the addition of bacteriophage concentrates *T2* or high-titred broth cultures *T5* to 100 ml. aliquots of tryptone broth cultures of *E. coli* B (at least 3 days old) at 37° C. The cultures then contained approximately 25 infective phage particles per bacterium. After 1–2 min., formalin was added to inactivate both phage and bacteria. After 3 hr. at room temperature, the bacteria with attached phage were centrifuged in the cold. When *T2* was used, the bacteria formed a highly viscous residue which was washed twice with saline. This viscosity was due to the deoxyribonucleic acid in the residue. Digestion of this acid was accomplished by the addition of 10 ml. of 0·15 *M* sodium chloride solution, 0·1 ml. of 0·028 *M* magnesium sulphate solution and 10 μgm. of deoxyribonuclease (Worthington Biochemical Corp.). The enzyme was allowed to act overnight at room temperature. This treatment was sufficient to allow resuspension of the bacteria with attached phage, which were sedimented by centrifugation prior to the addition of serum. With *T5*, the bacteria formed a residue easily resuspended in saline. Digestion with deoxyribonuclease was therefore not required. The *E. coli*-*T5* complex was washed three times with saline prior to the addition of serum. As a control, *E. coli* cells without phage were similarly treated with formalin and saline. Each serum (10 ml.) was adsorbed twice with either the *E. coli*-*T2* or *E. coli*-*T5* preparations for 1 hr. at 0° C. An aliquot was similarly treated with the control *E. coli* cells.

Phage-neutralizing activity titres of adsorbed and unadsorbed sera using homologous and heterologous bacteriophage are summarized in Table 1. Adsorption of normal serum with *E. coli*-phage complex resulted in almost complete removal of phage-neutralizing activity when tested against the homologous phage. In contrast, when this adsorbed serum was titrated against heterologous phages in the *T*-series, phage-neutralizing activity was essentially equal to that of the unadsorbed serum. This indicates that the component essential for the neutralization of phage by normal serum is a substance characterized by marked specificity. It is also interesting to note that the serum adsorbed with *T2* phage and titrated against *T4* and *T6*, both serologically related to *T2*[6], had about 50 per cent of the phage-neutralizing activity of the untreated serum. Conversely, the same serum, when tested against serologically unrelated phage, showed only slight diminution of activity as compared with the neutralization by the untreated serum.

Table 1. Neutralization of Homologous and Heterologous Bacteriophage by Adsorbed and Unadsorbed Normal Human Serum

Serum No.	Phage-neutralizing activity titre* against: *T2*	*T4*	*T6*	*T5*	*T7*
120858 Untreated	23	7	65	10	16
120858 Adsorbed with *E. coli*-*T2*	<1	3·3	37	9·5	15
20959 Untreated	22	11	51	16	25
20959 Adsorbed with *E. coli*-*T5*	19	6	46	<1	23

* The reciprocal of the amount of serum (ml.) required to neutralize 50 per cent of about 10^8 phage particles in a total volume of 1 ml. at 37° C. for 1 hr. Serum and phage were diluted in veronal buffer containing 0·1 per cent bovine serum albumin (ref. 2).

Table 2. Complement, Properdin and Phage-Neutralization (PNA) Titres of Unadsorbed and Adsorbed Normal Human Serum

Human sera	Serum No. 120858 PNA titre against *T2*	Complement units*	Properdin units†	Serum No. 20959 PNA titre against *T5*	Complement units*	Properdin units†
Untreated	23	240	12	16	230	12
Adsorbed with *E. coli* cells	19	216	4	14	230	6
Adsorbed with homologous *E. coli*-*T* phage complex	<1	180	4	<1	192	6

* The reciprocal of the dilution of serum required to hæmolyse 50 per cent of the sensitized sheep erythrocytes ($1·5 \times 10^8$ cells) in a total volume of 1·5 ml. at 37° C. for 30 min. Triethanolamine buffered saline was used as diluent.

† Euglobulin precipitates dissolved in endpiece were assayed as described (ref. 8).

Studies of the adsorbed and unadsorbed sera were undertaken to characterize further the serum components responsible for this activity against phage. Since the properdin system has been suggested as the factor in normal serum required for phage neutralization[7], it was necessary to measure the complement- and properdin-levels in adsorbed and unadsorbed sera. The complement titres in Table 2 indicate a slight loss in hæmolytic complement as a result of the adsorption. The slight reduction in phage-neutralizing activity against heterologous phage types seen in Table 1 may be related to this complement loss. From the properdin and phage-neutralizing activity titres in Table 2, it can be seen that adsorption with *E. coli* cells alone resulted in a 2–3 fold reduction in properdin with a scarcely detectable decline in phage-neutralizing activity. The results in Table 2 also indicate that the attached phage particles are not responsible for any detectable loss of properdin. Of greater significance, however, the phage-neutralizing activity is markedly reduced in the serum adsorbed with homologous phage despite the presence of the properdin system components. Only very slight reduction in activity occurred in the serum adsorbed with *E. coli* cells alone. Yet the properdin-levels in both adsorbed sera were the same and were within normal limits. The results indicated that sera adsorbed with homologous phage but having components of the properdin system were incapable of phage neutralization. Normal antibody, removed by adsorption with the homologous phage, appeared indispensable.

The origin of normal antibodies to phage, or to any microbe, is not readily determined. That some normal antibodies are specific does not necessarily favour any particular theory concerning their origin. It merely means that they react with certain organisms, and not with others, because of a chemical correspondence of certain specific combining groups. The pooled sera of 6 germ-free 6-week-old guinea pigs possessed greater phage-neutralizing activity against *T2* (titre equal to 33) than the sera of conventional guinea pigs (titre equal to 18) of the same strain and age. Hæmolytic complement- and properdin-levels of the two pools did not differ. Of course, germ-free animals are not necessarily free from viruses and are subjected to a variety of antigenic materials from sterile foods and viruses which may possess antigenic groupings cross-reactive with those of the *T*-phages. Thus, the possibility that phage-neutralizing activity

resulted from antigenic stimulation in these germ-free animals cannot be excluded. The globulin-levels of the germ-free animals tested were considerably below normal, however, and the gamma fraction was scarcely detectable by paper electrophoresis.

Properdin has been described as a single substance capable of neutralizing viruses and destroying certain abnormal red cells and bacteria[9]. The results of our experiments indicate that normal antibodies of marked specificity, rather than properdin or other non-specific substances, are essential requirements for phage-neutralizing activity. In the presence of antibody, properdin may conceivably augment the existing phage-neutralizing activity.

An experiment by Bordet[10] in 1899 led to a similar conclusion. The question was raised at that time whether the activities of normal serum could be explained by one substance, or a few substances. Bordet adsorbed normal horse serum with cholera vibrios, and after removal of the cholera vibrios with attached agglutinins by centrifugation the serum lost its ability to agglutinate cholera vibrios but clumped typhoid bacilli as intensely as the unadsorbed serum.

We wish to thank Drs. M. Forbes and S. Formal for the germ-free animal sera obtained from the Germ-free Research Project, Walter Reed Army Institute of Research. We also wish to thank Mr. W. Spilman of the Division of Immunology, Walter Reed Army Institute of Research, for his suggestions in the preparation of concentrated *T*2 phage suspensions.

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Spermine as a Protective Agent against Osmotic Lysis

PREVIOUS studies have revealed a close relationship between the growth requirement of *Pasteurella tularensis* and *Neisseria perflava* for spermine or allied polyamines and their marked osmotic fragility[1,2]. The susceptibility of those bacteria to the osmotic environment is manifested by leakage of cell material, rapid decay of respiratory ability and a sharp decline of viability following their brief exposure to distilled water. In the case of *P. tularensis* the osmotic cell damage is largely prevented by microgram amounts of spermine. On the other hand, the dependence of *N. perflava* on spermine as an essential growth factor in a chemically defined medium can be eliminated by proper adjustment of its tonicity.

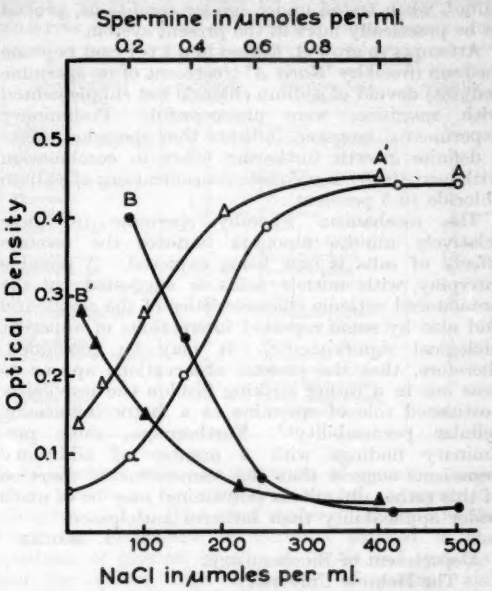

Fig. 1. Comparison of the protective effect of sodium chloride and spermine against osmotic lysis of *Achromobacter fischeri*. Washed cells of *A. fischeri* (about 3 mgm. protein content) were suspended in 1-ml. amounts of a 3 per cent sodium chloride solution supplemented with spermine in amounts corresponding to those indicated in the figure. At the end of 15 min. incubation the cells were spun off and resuspended in 5-ml. samples of the various sodium chloride or spermine solutions tested. After additional 30 min. incubation the extent of lysis was determined by measuring the turbidity of the suspensions and the absorbance of the supernates following centrifugation. Incubation temperature, 28° C. Curves *A*, *A'* and *B*, *B'* represent turbidity of the suspensions at 550 mμ and absorbance of the supernates at 260 mμ in the various sodium chloride and spermine solutions respectively (see text)

The present communication reports on the ability of spermine to prevent osmotic lysis in the obligate halophilic species, *Achromobacter fischeri* (A.T.C.C. 7744).

This organism is perfectly stable when suspended in the standard 0·5 *M* sodium chloride solution. It starts disintegrating, however, as soon as the osmotic strength of the suspending medium is diminished down to a critical level of about 0·25 *M* sodium chloride, below which the extent of cytolysis progresses in a roughly proportional manner to the decrease of osmolarity (Fig. 1). The cell rupture is evidenced by a drop in the turbidity of the suspension (curve *A*), as well as a concomitant increment in the absorbance of the cell-free supernate following centrifugation (curve *B*). The rise in optical density of the supernate is obviously attributable to leakage of cell constituents and the strong maximum exhibited close to 260 mμ is indicative of a large share of nucleotidic material.

The drastic lysis of *A. fischeri* which occurs in distilled water could be completely suppressed by adding 0·0005 *M* spermine provided that the cells have been pre-incubated with a similar amount of spermine while suspended in the standard sodium chloride solution. Graded amounts of spermine afforded, within a certain range of mounting concentrations, increasing protection against osmotic lysis, a definite effect being still perceptible with a quantity as low as 5 μgm./ml. (curves *A'*, *B'*). Putrescine and spermidine, known biosynthetic precursors of sper-

mine[3], when tested under similar conditions, proved to be practically inert in the present system.

Attempts to grow *A. fischeri* in a 1 per cent peptone medium (freed by 'Norit *A*' treatment of its spermine content) devoid of sodium chloride but supplemented with spermine, were unsuccessful. Preliminary experiments, however, indicate that spermine exerts a definite growth furthering effect in combination with osmotically inadequate concentrations of sodium chloride (0·5 per cent).

The mechanism whereby spermine in these relatively minute amounts imitates the osmotic effects of salts is now being explored. A possible interplay with nucleic acids is suggested by the pronounced cationic characteristics of the compound and also by some reported interactions of apparent biological significance[4,5]. It may be concluded, therefore, that the present observations appear to bear out in a rather striking fashion the previously postulated role of spermine as a factor influencing cellular permeability[1,2]. Furthermore, some preliminary findings with a number of additional organisms suggest that the 'osmomimetic' function of this rather ubiquitous polyamine[6] may be of much wider applicability than hitherto anticipated.

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Phagocytosis of *Escherichia coli* Protoplasts

This communication reports the results of experiments on phagocytosis of the bacterial protoplasts prepared from *Escherichia coli* B, and on phagocytosis of bacterial vegetative forms obtained from these protoplasts by reversion.

The protoplasts were prepared by Lederberg's penicillin method[1]. Before each experiment the protoplasts were washed free of saccharose with physiological saline.

Both protoplasts and vegetative forms of *E. coli* were used for phagocytic tests made with horse leucocytes. The phagocytic reactions were carried out in various media, namely, in fresh undiluted serum, in fresh diluted (1 : 10) serum, in undiluted serum inactivated for half an hour at 56° C., and in inactivated diluted serum (1 : 10). The phagocytic indices were thus determined, both in the presence and in the absence of thermolabile components of complement. Such experiments afford an opportunity for discovering possible differences in the degree of phagocytosis of normal vegetative forms and of protoplasts, depending on the presence or absence of complement and fresh serum. The diluting of serum was necessary to avoid inhibition of the degree of phagocytosis. Such inhibition occurs in the phagocytic reaction with *E. coli*.

The phagocytic indices were determined by the method of Skurski *et al.*[2] without prior sensitization; the time of phagocytosis was 20 min. Comparison of the phagocytic indices afforded a measure of the phagocytosis of both vegetative forms and protoplasts. According to Wright's definition[3], the phagocytic index is the average number of bacterial cells phagocytized by one leucocyte (100 leucocytes were counted in each preparation).

The results are presented in Table 1. They show the existence of large differences in the degree of phagocytosis, the phagocytic indices obtained with protoplasts being significantly lower than those obtained with mother cells. Photomicrographs showed that protoplasts within leucocytes (phagocytized protoplasts) were swollen, whereas the shape of protoplasts outside the leucocytes were similar to those in control preparations.

Table 1. Phagocytosis of Protoplasts and Normal Forms of *E. coli* B, and of *E. coli* B Cells obtained by Reversion of Protoplasts

Phagocytized cells	Phagocytic indices			
	Normal serum		Inactivated serum	
	Undiluted	Diluted 1 : 10	Undiluted	Diluted 1 : 10
E. coli B (normal forms)	12·0	19·3	23·2	13·9
Protoplasts	1·9	5·0	9·5	4·7
Protoplasts after 6 hr. incubation	11·0	22·0	24·4	10·0
Protoplasts after 24 hr. incubation	10·0	19·0	23·3	9·5

Lederberg[1] reports that in favourable conditions reversion of protoplasts to the mother forms may occur. We have investigated this phenomenon in relation to phagocytosis. Reversion occurred in broth at 37° C. It is evident from Table 1 that after 6 hr. incubation of the protoplasts at 37° C. in broth, the phagocytic indices returned to the values obtained in control tests with mother cells. The differences in the values of the phagocytic indices of vegetative forms and of protoplasts were independent of the medium in which the phagocytic reaction occurred.

The protoplasts were much less readily phagocytized both in the presence of fresh serum (containing all the active components of complement), and in inactivated serum (containing inactivated thermolabile components of complement).

According to Park and Strominger[4], penicillin inhibits the biosynthesis of bacterial cell walls giving the protoplasts. The present communication indicates that the removal of cell walls leads to significant inhibition of the phagocytic reaction.

Prolongation of incubation times up to 24 hr. failed to change the phagocytic indices. Typical vegetative forms were observed after incubation for 24 hr., whereas after 6 hr. incubation both vegetative forms and protoplasts appear. Neither reversion nor phagocytic indices changes were observed with protoplasts stored at 2° C. in physiological saline and 20 per cent saccharose for several days.

This investigation is being continued, and the details will be published elsewhere.

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Production of Bacterial Mutants with Nitrous Acid

RECENTLY, Mundry and Gierer[1] used nitrous acid to produce mutants of tobacco mosaic virus by chemical alteration of its ribonucleic acid, as demonstrated by Schuster and Schramm[2]. This communication deals with the mutagenic effects of nitrous acid on living cells of the bacterial species *Escherichia coli* and *Salmonella typhimurium*, the genetic substance of which is deoxyribonucleic acid.

Nitrous acid inactivates bacterial cells, reducing the ability of a suspension to form colonies. In the experiments described here, the rate of inactivation varied with differences in the state of metabolism of the cells. Those with active metabolism gave higher rates of inactivation per unit time than cells which were in a resting phase. The inactivation of such resting cells, which have only one nucleus, followed a double-hit curve (Fig. 1). Starting with about 10^8 cells per ml. its slope was tested for four logarithmic cycles of the ordinate.

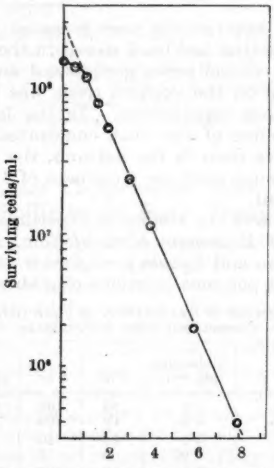

Fig. 1. Sensitivity of resting cells of *E. coli*, strain *B*, to 0·017 *M* nitrous acid (*p*H 4·5)

Wild-type cells of *E. coli*, strain *B*, which had survived treatment with nitrous acid, were plated on nutrient agar so as to give about 150 colonies per plate. Their synthetic abilities were tested by replica-plating[3] the colonies on minimal medium. Since this medium contained only glucose and inorganic salts, it supported growth of the wild-type strain but inhibited the multiplication of cells which had mutated to auxotrophy. Using this technique, auxotrophic mutants induced by treatment with nitrous acid were found. By means of a method published elsewhere[4] the mutants were tested for their growth requirements.

Details of the experiments and conclusions drawn are as follows.

(1) Nitrous acid is a potent mutagen: with an inactivation-rate of 10^{-5} about 4 per cent of surviving cells were auxotrophic mutants.

(2) The spectrum of mutants (Table 1) induced by nitrous acid differs significantly in some respects from that observed after induction with ultra-violet, other mutagens or after spontaneous origin.

Table 1. DISTRIBUTION PATTERN OF NUTRITIONAL REQUIREMENTS FOUND IN AUXOTROPHIC MUTANTS INDUCED BY INCUBATION OF WILD-TYPE CELLS OF *E. coli*, STRAIN *B*, WITH NITROUS ACID

Nutritional requirements tested	No. of mutants found	Nutritional requirements tested	No. of mutants found
Adenine	21	Proline	11
Adenine or guanine	2	Proline or glutamic acid	1
Guanine	4	Serine	2
Cytosine	0	Threonine	9
Cytosine or uracil	17	Tryptophane	28
Thymine	2	Tyrosine	9
Arginine	55	Valine	17
Aspartic acid	2	Ascorbic acid	0
Cysteine	30	Nicotinic acid	13
Cysteine or methionine	10	Pantothenate	0
Glutamic acid	3	Pyridoxal-phosphate	2
Glycine	4	Pyridoxal-phosphate or glutamic acid	1
Glycine or serine	2	Riboflavin	0
Histidine	37	Riboflavin or nicotinic acid amide	3
isoLeucine	9	Other unanalysed requirements	224
isoLeucine or valine	1		
Leucine	15		
Lysine	10		
Methionine	23		
Phenylalanine	1		

It is identical with the spectrum of mutants found after induction by disintegration of incorporated phosphorus-32 [4]. The most striking features are the very rare occurrence of mutants blocked in the synthesis of glycine, glycine or serine and cysteine; and the rather high frequency of purine- and pyrimidine-less mutants mainly of the cytosine- or uracil-less type as well as of the argine-less type.

(3) The production of auxotrophic mutants in the range tested (4 per cent of surviving cells were auxotrophs) follows a double-hit curve (Fig. 2).

(4) When suspensions are used the cells of which are in different metabolic states, the percentage of mutants induced by nitrous acid is independent of

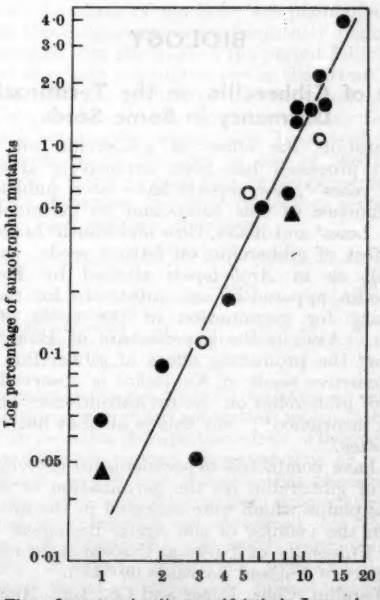

Fig. 2. Relationship between the percentage of induced mutants and the time of incubation with 0·017 *M* nitrous acid (*p*H 4·5). Since the plot is a straight line with a slope of about 60° when both axes have the same logarithmic scale the curve is evidence for a double-hit reaction. ●, Resting cells of *E. coli*, strain *B*; ○, cells from a growing culture of *E. coli*, strain *B*; ▲, resting cells of *Salmonella typhimurium*, strain *LT* 2

the rate of inactivation (Fig. 2) and depends only on t. Thus the rate of mutation is not influenced by the metabolic state of the cell. This is consistent with the hypothesis that the mutational effect of nitrous acid on living bacteria is direct, that is to say, due to the conversion of adenine, guanine and cytosine present in the deoxyribonucleic acid into hypoxanthine, xanthine and uracil.

(5) From 600 auxotrophic colonies which arose from bacteria plated directly after contact with nitrous acid, none was sectored even with survival rates higher than 5×10^{-1}, nor did any contain wild-type cells. This is contrary to the results of work[4] with phosphorus-32 incorporated into living bacterial cells; in which case the mutagenic effect of disintegrating phosphorus-32 gave cells which formed colonies composed of auxotrophic and prototrophic sectors. The lack of sectored colonies after treatment with nitrous acid, together with the double-hit type of the mutation curve, leads to the conclusion that in order to cause a mutation which is detectable under the experimental conditions applied, nitrous acid must affect both strands of the double-helix of the deoxyribonucleic acid molecule.

I am much indebted to Prof. G. Schramm and Dr. H. Schuster for discussions of this work, a detailed account of which is in preparation.

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BIOLOGY

Effect of Gibberellin on the Termination of Dormancy in Some Seeds

Although the effect of gibberellin on certain growth processes has been extensively studied in recent years[1,2], few reports have been published on the influence of this compound on germination of seeds. Lona[3] and Kahn, Goss and Smith[4] have shown the effect of gibberellin on lettuce seeds. In these, as well as in *Arabidopsis* studied by Kribben[5], gibberellin apparently can substitute for red light necessary for germination of the seeds of these species. Also in the experiments of Bünsow and Bredow[6] the promoting effect of gibberellin on the light-sensitive seeds of *Kalanchoë* is observed. The effect of gibberellin on the revitalization of old seeds is also mentioned[2]; but this is all that has come to our notice.

We have conducted experiments to determine the effect of gibberellin on the germination of seeds of various plants which were collected in the autumn of 1958 in the vicinity of the Arctic Biological Station of the University of Turku at Utsjoki in the northernmost part of Finland (latitude 69° 45′).

Gibberellin (Chas. Pfizer and Co., Inc., New York) was found to exert a marked influence on the germination of the seeds of eight of about the 50 species examined. These eight species are *Luzula parviflora*, *Trollius europaeus*, *Erysimum hieraciifolium*, *Draba hirta*, *Geranium silvaticum*, *Diapensia lapponica*, *Gentiana nivalis* and *Bartsia alpina*.

The germination experiments were performed on filter paper in a Johnson germinator at a temperature of 24° C., and in diffuse daylight conditions.

Because the effect of gibberellin was first observed in the experiments on *Gentiana nivalis* seeds, these have been studied most thoroughly to date. In October 1958 not a single one of 500 seeds germinated in the control experiments (that is, on filter paper), and light as well as cold treatment of 20 days duration had no effect. The results obtained when the seeds were exposed to the action of gibberellin are shown in Table 1. In these conditions the germination took place on the sixth day (Table 1).

Table 1. The Effect of Gibberellin on the Germination of *Gentiana nivalis*. (Percentage Germination)

Time of exposure	Gibberellin (per cent)				
	0·5	0·25	0·1	0·01	0·001
1 hr.	100	100	100	1	0
3 hr.	100	100	100	4	0
6 hr.	100	95	97	45	0
8 hr.	90	92	91	70	6
24 hr.	80	95	90	76	22

When the experiments were repeated in February 1959 on seeds that had been stored in the laboratory, none of the control series germinated and the effect of gibberellin on the exposed seeds was the same as in the autumn experiments. In the later part of March the effect of the weak concentrations 0·01 is more effective than in the autumn, the germination percentage being after the treatment of 3 hr. as much as 85 per cent.

Table 2 shows the results of experiments in which the seeds of *Erysimum hieraciifolium*, *Draba hirta*, *Bartsia alpina* and *Luzula parviflora* were exposed to 0·1 and 0·01 per cent solutions of gibberellin.

Table 2. Percentage of Germination of Four Different Species Pretreated with Gibberellin

	Gibberellin (per cent)	3 hr.	6 hr.	24 hr.	Control
Erysimum hieraciifolium	0·1	85	100	100	25
	0·01	90	100	100	
Draba hirta	0·1	16	20	95	0
	0·01	5	5	10	
Bartsia alpina	0·1	10	80	90	5
	0·01	10	45	85	
Luzula parviflora	0·1	10	10	90	10
	0·01	5	15	90	

In the case of *Trollius*, prolonged soaking in gibberellin solution is necessary to effect germination. After 24 hr. 15 per cent of the seeds exposed to 0·1 per cent gibberellin and 5 per cent of seeds exposed to 0·01 per cent gibberellin germinated, whereas after 48-hr. treatment the respective percentages were 90 and 80.

It is obvious that gibberellin penetrates into the embryo of the *Trollius* seed very slowly. A preliminary treatment with sulphuric acid therefore was given. Seeds pretreated with sulphuric acid for 20 min. germinated to the extent of 70 per cent when they were exposed to a 0·01 per cent gibberellin solution for 3 hr.

The germination experiments with *Diapensia lapponica* were carried out in February 1959. 15 per cent of the seeds exposed to a 0·1 per cent gibberellin solution for 24 hr. were germinated whereas none of the seeds of the control series germinated.

A combination treatment with sulphuric acid and gibberellin gave the best results with *Geranium silvaticum*. The seeds stored in normal room con-

dition germinated very poorly, the germination being less than 1 per cent. Exposure to gibberellin solutions had no effect, and exposure to concentrated sulphuric acid only a slight effect. When, however, the acid-treated seeds were exposed to the gibberellin solution, a very high proportion of the seeds germinated. The most favourable procedure was to treat the seeds first with concentrated sulphuric acid for 30 min. and, after washing, to 0·1 per cent gibberellin for 6 hr. Prolongation of the latter period of exposure to 24 hr. did not increase the proportion of germinated seeds.

No correlation between systematic relations and the effect of gibberellin was established. Seeds of *Gentiana uliginosa* and *G. amarella* from southern Finland and *Luzula sudetica*, *L. pallescens*, *L. spicata*, *L. multiflora* and *Draba incana* did not respond to the treatment with gibberellin. Strains of *Erysimum hieraciifolium* from southern Finland and Poland did not differ significantly from the strains from northern Finland.

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Some Unusual Outgrowths from Secondary Follicles in Soay Sheep

Some 'plugs' of undifferentiated cells were discovered while the secondary : primary follicle ratio was being determined in preparations of skin from four six-month old Soay lambs. The plugs were found at the sebaceous gland level, and were traced towards the skin surface through the series of sections in order to determine whether they were developing follicles, shedding follicles, or parts of sebaceous glands.

In fact, each plug joined on to the ectal side of a secondary follicle immediately beneath the epidermis and thus arose as an outgrowth. They are thought to be follicle branches that have occurred during the development of follicles[1]. But they were also found in two adults, 3 and 4 years old. The original samples were taken in September 1958 ; and skin obtained later when two of the lambs were slaughtered unfortunately did not give sufficiently good preparations to show these outgrowths. But a third adult, two years old, sampled in February 1959, had them.

Four of the six outgrowths traced in detail had a lumen extending 60–80μ below their attachment (Fig. 1). This joined the follicle lumen and was either lined or blocked with stratum corneum in the upper part, so that here the outgrowth resembled the sweat duct of a primary follicle. Below this the lumen was blocked discontinuously with pigment. On the termination of the lumen at the sebaceous gland level of the parent follicle the cells of the outgrowth became undifferentiated, usually on the side adjacent to the follicle, but on the opposite side they had the appearance of sebaceous cells for 25–60μ. One outgrowth (in an adult) in fact had an outgrowth of sebaceous tissue in the shape of a sebaceous gland. Below this the cells appeared undifferentiated for the remaining 80–100μ of the length of the outgrowth. Another outgrowth, however, had a bud of undifferentiated cells immediately below the sebaceous portion.

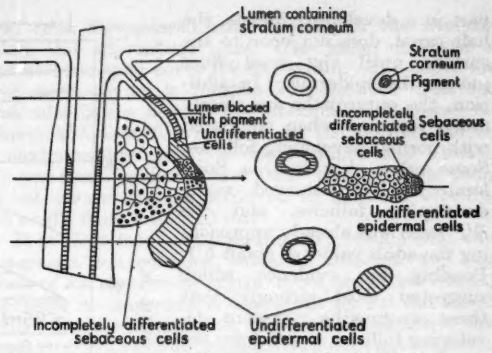

Fig. 1. Left, diagram of outgrowth reconstructed from a series of tracings of cross-sections made with a projection microscope; right, some of the cross-sections

One outgrowth (in a lamb) was apparently at an early stage of development in that the cells had an undifferentiated appearance over the whole of its length, and the lumen appeared discontinuously in short lengths. Another (in an adult) was apparently more advanced because it had fragments of inner sheath in the lumen, but there were no traces of fibre.

Most of the outgrowths were apparently attached to the sebaceous gland and sometimes to the outer sheath of the parent follicle, and one was attached to the outer sheath of another follicle. This attachment does not seem to be close, however, but has arisen only from the contact of undifferentiated tissue. At the level of the contact with the sebaceous gland the undifferentiated tissue was inconspicuous, being confined to a narrow strip at the edge of the gland, and in horizontal section there was little to show that the sebaceous gland was other than normal.

Most of the fibres of the Soay are pigmented, and although the outgrowths were frequently pigmented it was noticed that the fibre of the parent follicle was often the only non-pigmented one in the group. This suggested that the growth of the fibre had become less vigorous. The percentage of secondary follicles with these outgrowths ranged from 1 per cent in one of the adults to 23 per cent in one of the lambs. From Table 1 it will be seen that as the percentage of outgrowths decreases, the ratio of mature secondary follicles to each primary increases.

Table 1

	Lambs				Adults	
Percentage of secondary follicles with outgrowths	31	27	20	10	6	1
Mature S/P ratio (S_F/P_F)	2·47	2·61	2·99	2·88	3·6	4·64

These outgrowths are thought to have no relation to the outgrowths called capsules[4], which are now thought to be milia (benign tumours). Their appearance near the skin surface suggested that they were sweat glands, and it was thought that their parent follicles may have been intermediate between primary and secondary follicles as described by Burns[2]. However, the outgrowths bear much resemblance to developing follicles ; they are very similar in appearance to the developing follicle shown in ref. 3, Fig. 23, and they are on the same side of the follicle as the follicle branches of Hardy and Lyne.

The chief way in which they differ from the developing follicles in fœtal skin of the Romney breed[4] is that the lumen joins the follicle lumen and contains much stratum corneum. The comparable

part in a developing follicle, the hair canal, does not open to the surface until the wool fibre pierces the epidermis. In addition, the outgrowths are clearly much retarded when compared with normal developing follicles. Some other skin from a Soay lamb at birth showed many developing follicles, and the S/P ratio was already approaching the adult value[5] of about 5/1. Possibly the evidence which suggested most strongly that these outgrowths represent developing follicles comes from the trend in Table 1, in which the S/P ratio of follicles with fibres increases as the proportion of outgrowths decreases.

The attachment of the outgrowth to its parent's sebaceous gland seems to be abnormal. But this may throw light on the development of sebaceous tissue because it was always at the level of the parent's sebaceous gland that the cells of the outgrowth became sebaceous-like. It may be that here the undifferentiated cells are under the influence of an organizer from the parent's sebaceous gland which leads to their development into sebaceous cells.

I wish to thank Prof. T. Grahame of the Royal (Dick) Veterinary College, Edinburgh, for providing the material for this study, and Miss M. M. Vickers and Miss R. G. Jasper for their technical assistance.

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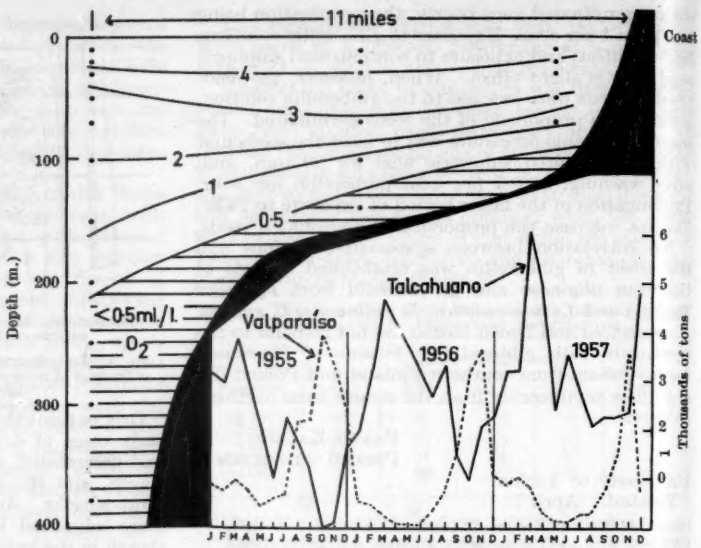

Fig. 2

Fig. 1

Relationship between the Hake Fishery and a Southerly Sub-surface Return Flow below the Peru Current off the Chilean Coast

A combined hydrographic and biological investigation has been launched jointly by the Department of Fish and Game of the Ministry of Agriculture of the Republic of Chile and the Technical Foreign Aid Program of the Federal Republic of Germany in order to find possible relationships between the migration and behaviour of fish and the changing hydrographic conditions off the Chilean coast.

The hake, *Merluccius gayi gayi* (Guichenot), is the most important fish to the economy of Chile. More than 50 per cent of the total landings of all species consists of hake. The main trawling grounds are the banks at a depth of 100–150 metres between Papudo (lat. 32° 30′ S.) and the Gulf of Arauco (lat. 37° 15′ S.). Since the continental shelf is very narrow along the Chilean coast, the hake fishery takes place within 10 miles of the shore line. The fish are caught mainly off Valparaiso, San Antonio and Talcahuano. In the northern zone of Valparaiso area, the best fishing occurs during the months September–December, with a steep maximum in the catch statistics in October or November. A sharp drop of the catches to almost zero follows after December. In the Talcahuano area we find moderate catches throughout the year but a pronounced maximum in March (Fig. 1). The yield in the San Antonio area is intermediate. It seems that the stock of hake migrates successively from the northern area of Valparaiso southward, and later in September back to the north.

A preliminary hydrographic survey, carried out off Valparaiso in February 1959, shows extremely interesting results with regard to the oxygen of the water which covers the hake fishery banks (Fig. 2). The oxygen content of the water in an intermediate layer between 100 and more than 380 m. depth (possibly deeper, but no more wire was available) is between 1·0 and 0·4 ml./l., which suggests that the hake has been pushed southward by the lack of oxygen in the water.

By means of salinity and temperature sections off Peru and northern Chile, Gunther[1] proved the existence of a sub-surface return flow below the Peru Current at a depth between 200 and 400 m. Since plankton production in the Peru Current is very high[2], the decomposition of organic material, which sinks into the return flow beneath, apparently causes great depletion of oxygen in this layer. Similar conditions have been found in the north-east Pacific[3].

In Chilean waters there is only Gunther's work, based on the temperature and salinity observations obtained during the cruise of the *William Scoresby* in 1931. We do not know how far this oxygen-poor water extends to the south, how far offshore it extends to the west, and how deep it is. According to the salinity sections drawn by Gunther[1], it seems that this sub-surface current is pushed towards the coast by the effect of the Coriolis force. By changes of some kind in the hydrographic or meteorological conditions, this water covers the hake-fishery banks at certain times of the year, affecting the migration of the hake,

and consequently determining the yield of the fishery in the three zones.

The investigation will be continued and a detailed report published later elsewhere.

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Life-Span of Rat and Mouse Erythrocytes in the Rat–Mouse Chimera

THE secondary disease observed in irradiated animals treated with foreign bone marrow is of general interest and importance. The basic question which remains unanswered is whether immunologically competent cells in the chimera originate from the donor, the host, or a combination of both. Erythrocyte life-span studies might be expected to throw light on whether this secondary reaction, in so far as it is reflected in red cell survival, is of host or of donor origin.

Nine $(C57 \times 101)F_1$ female mice that survived 60 days after 900 r. of X-rays (*LD*100/20 days) and an injection of Sprague–Dawley rat bone marrow (55×10^6 nucleated cells/mouse) on day zero were transfused intravenously with 0·2 ml. of a 45 per cent suspension of chromium-51-labelled red blood cells in saline. Four mice were given labelled isologous erythrocytes and five were given labelled rat erythrocytes; blood was pooled from normal donors for each group. The activity of chromium-51 in circulating blood measured at 24 hr. was taken as 100 per cent, and measurements were made at intervals for 7 weeks. 25 days after the transfusion of labelled cells these animals had essentially 100 per cent rat erythrocytes by the mannitol test[1].

The level of activity of chromium-51 is plotted against time in Fig. 1, and it can be seen that the rat cells disappeared more rapidly in the chimera than did the mouse cells. The latter followed a normal life-span curve for this strain. The curve for rat erythrocytes is intermediate between that for rat cells in the normal rat (maximum life span: 60 days) and that for rat cells in the normal mouse (maximum life span: 4 days) (Smith and Tohá[2]). It was found by Smith and Tohá[2] that rat cells from a rat–mouse chimera survived in normal recipients like cells from a normal rat.

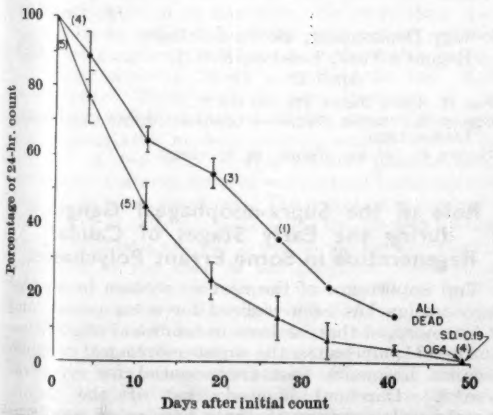

Fig. 1. Disappearance of chromium-51 activity from the circulating blood of rat–mouse chimeras transfused on day zero minus one with either mouse (upper curve) or rat (lower curve) labelled erythrocytes. Number of surviving animals in each group is shown in parentheses at three time points on the curves. Standard deviations are indicated by vertical bars at each point plotted

It is clear from these preliminary results that the long-term heterologous chimera, although 100 per cent of its circulating erythrocytes are of rat origin, provides an imperfect environment for rat red blood cells. On the other hand, the presence of active rat hæmatopoietic tissues in such a host does not influence the survival of transfused mouse erythrocytes. This study therefore provides no evidence of an active rat immune system. Whether the accelerated destruction of rat cells reflects a recovering host immune system, as is clearly indicated by the findings of Gengozian *et al.*[3], or only the foreign biochemical and physiological environment of these heterologous cells cannot be answered by these data.

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Assortative Mating and Reciprocal Difference in the Blue–Snow Goose Complex

THE lesser snow goose *Chen hyperborea* and the blue goose *Chen caerulescens* are maintained as good species by the standard taxonomic works[1] though the morphological difference between them is confined to colour of plumage. The geographical distribution of the two is different. The snow goose ranges through Siberia, western Canada, the Hudson Bay area and Baffin Island; west of Banks Island the populations are unmixed. To the east, however, most populations contain some blue geese and in the Bowman Bay area of Baffin Island there exist several populations in which more than 90 per cent of the birds are blue. Where the populations are mixed, interbreeding between the two occurs, and the progeny produced are viable, fertile[2] and phenotypically distinct[3]. This has led some authorities[4] to consider the two forms to be polymorphs of one biological species. This communication is concerned with observations made by one of us (F. G. C.) on breeding behaviour in the mixed Boas River population, and for convenience the terms blue and snow are used in a purely descriptive sense. Table 1 shows the observed and expected (random mating) distribution for three

Table 1. OBSERVED AND EXPECTED DISTRIBUTION OF DIFFERENT MATINGS IN A MIXED BLUE AND SNOW GOOSE POPULATION

Type of mating	Two snow birds	Two blue birds	One blue, one snow	Total
No. observed	373	64	83	520
No. expected	330·4	21·4	168·2	520

types of mating: (1) both snow birds; (2) both blue birds; (3) one snow and one blue bird in a random sample of 520 breeding pairs from the Boas River population in 1952.

The χ^2 value for the deviations from expected is very large, 133·45, which for 3 degrees of freedom indicates a probability of less than one in a thousand of obtaining such a result by chance alone.

This is strong evidence for the existence of positive assortative mating in mixed populations and this is in agreement with earlier work[5,6].

In our study, however, the reciprocal matings snow × blue and blue × snow were distinguished. Table 2 shows for the 83 mixed pairs the observed and expected distribution.

Table 2. Observed and Expected Distribution of the Two Reciprocal Types of Mixed Mating in a Mixed Blue and Snow Goose Population

Type of mating	Snow (♀) and blue (♂)	Blue (♀) and snow (♂)	Total
Observed	54	29	83
Expected	41·5	41·5	83

The χ^2 value for the deviations in this case is 7·52 for 1 degree of freedom. The probability that this is the result of chance alone is less than one in a hundred. We may feel, therefore, confident that the incidence of the two reciprocal matings is really different in this, and presumably in other mixed populations, and it is of some evolutionary interest to speculate as to possible reasons for this. One reason may be that the sex ratio is different in the two phases. There are no indications that this is in fact true, but a sex ratio disturbed in the direction of relative female preponderance in snow geese and in the direction of relatively more males in the blue geese might give the effect observed.

A more likely hypothesis is that the mechanism governing resistance to unlike-mate choice more readily breaks down in one type of pair formation than in the other. This might involve different levels of receptivity on the part of the two female types, different levels of sexual drive on the part of the two male types or both. Only a study of the long-term dynamics of the mating barrier would tell us what factors are involved, but from other evidence it is possible to infer what might be happening. The frequency of the blue phase is increasing in all known mixed populations and more populations are becoming mixed; in the Darwinian struggle for existence it seems that the snow phase is losing to the blue and what is observed at present is a transient polymorphism (which may, of course, later become balanced).

Among other things one component of the selective advantage enjoyed by the blue phase may be sexual in that blue males find it easier to obtain an unlike mate than do snow males. The situation is complicated by the fact that the majority of blue birds involved in mixed matings are white-bellied (that is, heterozygous at the principal genetic locus governing difference in colour). This then implies that hybrid males may mate more easily than pure blue males, and in an evolutionary sense this might be a factor in promoting a balanced polymorphic system in the species-complex but equilibrium is so far from being attained than further speculation upon this point seems unwarranted.

Whatever the mechanism underlying the reciprocal mating difference, it is clear that it depends upon factors other than those which are responsible for assortative mating as such, although this is in itself a matter of considerable interest. Further study should show how far mating preference is a result of family structure[4], imprinting[7], pleiotropy of colour genes or more complex genetic systems, and will, it is hoped, contribute towards a better understanding of the taxonomic problem.

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Hæmoglobin and the Habitat of the Harpacticoid Copepod *Elaphoidella gracilis* (Sars)

Hæmoglobin has only recently been found in free-living copepods. Munro Fox[1] found that certain mud-dwelling harpacticoids contained this respiratory pigment, while allied species living in moss or open water lacked it. The correlation between hæmoglobin and habitat can now be extended by the discovery of hæmoglobin in *Elaphoidella gracilis*, a species which inhabits burrows in decaying aquatic vegetation. The red pigment in this species is easily visible under the microscope, and its identity was established spectroscopically.

Elaphoidella gracilis is not very often recorded because of its burrowing habits. Gurney[2], writing of its seasonal occurrence, says "the capture of it is so capricious that nothing certain is known". Donner[3] regards it as a summer form, but I have found this species to be present and active in the Long Water at Hampton Court throughout the whole of the last winter.

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Role of the Supra-œsophageal Ganglion during the Early Stages of Caudal Regeneration in Some Errant Polychætes

The importance of the nervous system in annelid regeneration has been realized for some years[1], and it has emerged that, at least in lumbricid oligochætes and nereid polychætes, the supra-œsophageal ganglion secretes hormones that are essential for regeneration[2–4]. Durchon[2] showed that if the supra-œsophageal ganglion of three species of nereid is removed regeneration is retarded. Hubl[4] discovered that, in lumbricids, extirpation of the supra-œsophageal ganglion at the same time as a number of posterior segments is removed totally inhibits

posterior regeneration, but that if the brain is removed 24–48 hr. after the posterior segments are amputated then posterior regeneration proceeds at the normal rate. There is thus a brief critical period during which the brain exerts its influence in initiating regeneration. Since Durchon extirpated the brain of his animals 24 hr. after amputating the posterior segments, it is not clear if the difference between his results and those of Hubl represents a real difference between lumbricids and nereids, or if it is due to the difference in experimental procedure.

Durchon's experiments have been repeated on *Nereis diversicolor*[5], and it has been found that extirpation of the supra-œsophageal ganglion 24-hr. before amputation of a number of posterior segments totally inhibits the proliferation of new segments, though one-third of the animals regenerate a pygidium. If the ganglion is extirpated 72 hr. after amputation of the posterior segments, regeneration is retarded, but not prevented, and 16 per cent of the animals proliferated 1 segment during the 30 days the experiment was continued. During this time, control animals regenerated between 1 and 11 segments (average 4).

The events occurring in the regenerating tip, the brain and the blood vascular system during the five days following the loss of posterior segments have been followed in more detail in *Nephtys cirrosa* and *N. hombergi* (members of the Nephtyidae, a family of polychætes closely related to the Nereidae).

During this period the wound is closed and, after an initial stage of dedifferentiation, blastema-formation and regeneration begin. Between the first and second days, muscle cells begin to dedifferentiate, and there is an accumulation of cœlomocytes at the wound. Cell division does not occur until the second day and is then confined to the gut epithelium and to a few dedifferentiated mesenchyme cells. By the third day, mitoses are common in the preterminal part of the nerve cord, and it is not until the fourth day that mitoses occur in the tip of the nerve cord and in the epidermis.

Within 6 hr. of amputation of a number of segments, neurosecretory cells in nucleus *S*[6] of the supra-œsophageal ganglion become active. After about 48 hr. the secretory cell-nucleus becomes very angular, the cytoplasm of the cell is vacuolated and appears to be depleted of its secretion. No more than one or two cells on either side of the ganglion are involved. Cells in ganglionic nucleus *Z* show visible signs of secretory activity 24–48 hr. after the loss of the segments. These cells become depleted of their secretion within a further 24 hr., but others in the same ganglionic nucleus start to secrete and replace them. A few cells in this nucleus contain neurosecretory material even in worms that have regenerated 12 or more segments. The secretion produced by both sets of cells migrates along the axons and those from ganglionic nucleus *Z* run to the cerebro-vascular complex at the base of the ganglion where neurosecretory material is released into the blood vessels[7].

Neurosecretory material is taken up by blood corpuscles[7], and these increase enormously in number during the early stages of regeneration. Initially, no more than one or two can be counted in the blood vessel beneath the brain; their numbers begin to increase about 12 hr. after amputation, and by the fifth day nearly 70 can be counted in the same length of blood vessel. A comparable increase in the number of blood corpuscles can be detected in vessels near the regenerating tip.

Detailed accounts of this work will be published elsewhere.

R. B. Clark
M. E. Clark

Department of Zoology,
University of Bristol. April 25.

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The Proposed Biological Term 'Pheromone'

Karlson and Lüscher[1] have recently proposed the term 'pheromone' for a class of substances which, while resembling hormones in some respects, cannot be included among them because they are not of an endocrine origin. It is claimed that the latter part of the proposed word is derived from the Greek *hormōn* (which should properly be *hormān*), meaning 'to excite'. This is not so. It was apparently the intention of the authors to find a term which, while being derivable from *hormān*, bore little overt resemblance to the word 'hormone'. This they achieved by the improper expedient of dropping the letter 'r', thus mutilating the root of the Greek word. It should be a principle, when a scientific term is derived from classical origins, that the term must by its structure be traceable to those origins. In the case of the word 'pheromone' that principle is not observed: there is no structural clue to its derivation from *hormān*. Indeed, the only Greek verb (apart from *pherein*) which might conceivably be involved in its ancestry means 'to swear'.

It would be an etymological improvement to re-instate the missing 'r', making 'pherormone'. If this too closely resembles 'hormone', an alternative would be to abandon Greek and use the word 'transcitant', derived from the Latin *trans* and *citare* (to excite). The approximate meaning, as well as the derivation, of this word is readily deducible—an advantage not shared by 'pherormone'.

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[1] Karlson, P., and Lüscher, M., *Nature*, **183**, 55 (1959).

It was our aim to introduce, for a class of substances, a scientific term of international usefulness. Based on a clear definition, it should be a short word easily pronounced in many languages. This is not the case with 'pherormone'. It must be admitted that the derivation of 'pheromone' from *hormān* is questionable. We would therefore not insist on it; but we regard the ending 'mone' as a proper suffix which is used in hormone, gamone, termone and pheromone.

The alternative proposal 'transcitant' is regarded as unfortunate. Its translation would refer to a neurohumoral transmitter substance rather than to a pheromone. The exciting activity is only one of their possible actions. Furthermore, the parallelism to hormone, gamone, etc., is entirely lost.

P. Karlson
M. Lüscher

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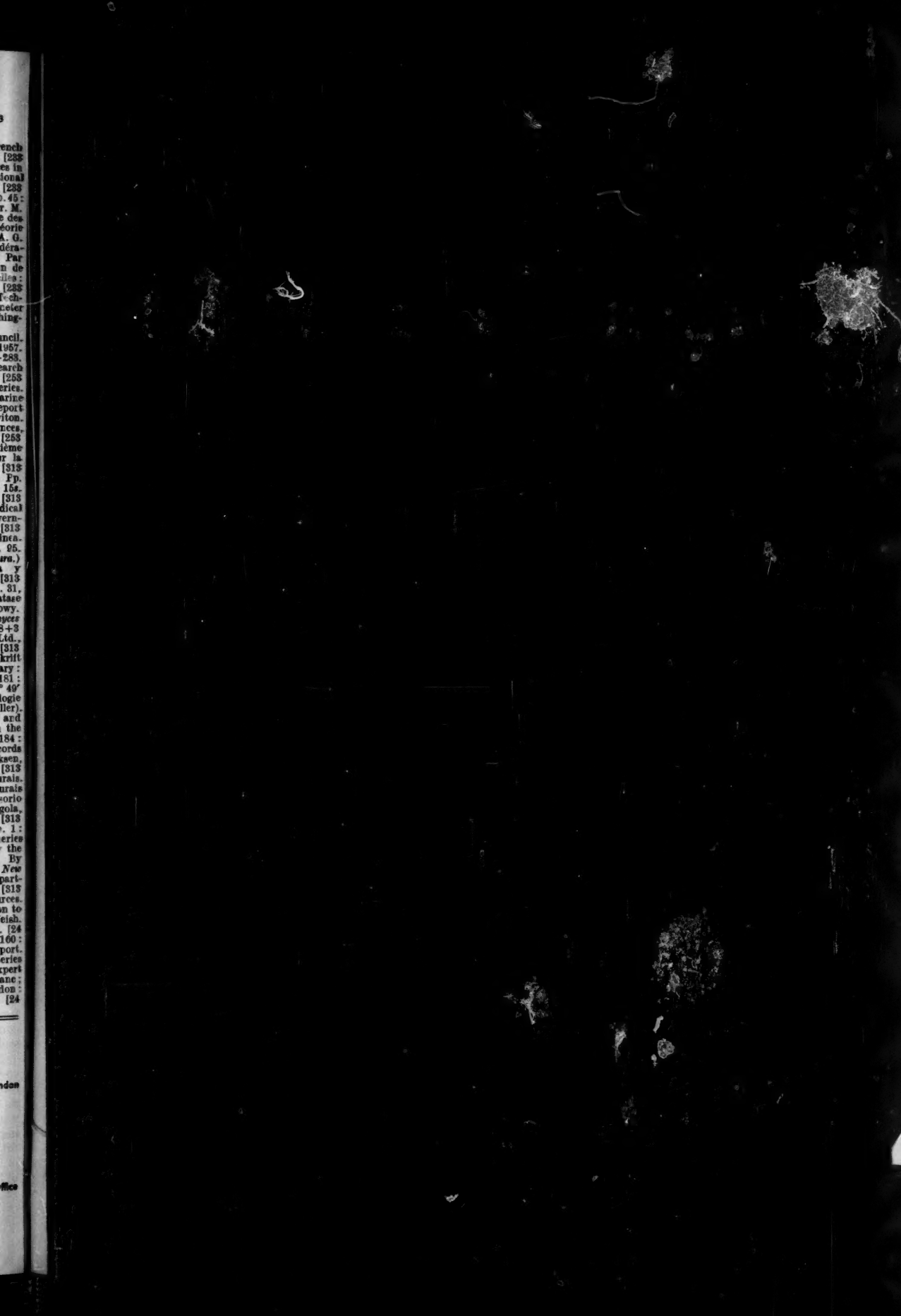
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